IJP (2019), Vol. 6, Issue 6 **(Research Article)**

Received on 29 May 2019; received in revised form, 21 June 2019; accepted, 23 June 2019; published 30 June 2019

NUTRITIONAL AND MICROBIAL ANALYTICAL STUDY OF VEDIC LIQUID ORGANIC MANURE CUM PESTICIDE KUNAPAJALA WITH DIFFERENT STORAGE TIME INTERVAL

Bishal Chakraborty [1], Indrajit Sarkar [*1], Soumen Maitra [1], Arpita Mandal Khan[1], Sekhar Bandyopadhyay [2] and Abhas Kumar Sinha [2]

Department of Floriculture, Medicinal and Aromatic Plants, Faculty of Horticulture [1], Department of Plant Pathology, Faculty of Agriculture [2], Department of Soil Science and Agricultural Chemistry, Faculty of Agriculture [3], Uttar Banga Krishi Viswavidyalaya, Pundibari, Coochbehar - 736165, West Bengal, India.

Keywords:

Kunapajala, Liquid organic manure, Storage time interval, Vedic manure and Vedic pesticide

Correspondence to Author:
Dr. Indrajit Sarkar

Professor and Head,
Department of Floriculture, Medicinal and Aromatic Plants, Faculty of Horticulture, Uttar Banga Krishi Viswavidyalaya, Pundibari, Coochbehar - 736165, West Bengal, India.

E-mail: indrajitsarkar_kpg@yahoo.co.in

ABSTRACT: A study on nutritional and microbial analysis of Kunapajala with different storage interval was conducted in the Department of Soil Science & Agricultural Chemistry and Department of Plant Pathology, UBKV, Coochbehar - 736165, West Bengal during March 2019. The motive of this work was to estimate the physicochemical properties, macro, and micronutrient content, and various microbial load of Kunapajala with a different storage time interval. Kunapajala was rich in N, P, K, Ca, Mg, S, Fe, Zn, Cu & Mn and it had a significant beneficial microbial load of Fungi, *Actinomycetes*, *Pseudomonas*, solubilising phosphorus bacteria (PSB), *Azotobacter*, *Azospirillum*, *Rhizobium* and *Trichoderma*. Nutrient content and microbial population change significantly with time in Kunapajala. So, continuous foliar and soil application of Kunapajala from the beginning and up to 40 days after preparation was beneficial to get maximum utilization. It is recommended that Kunapajala can be used as an alternative against chemical fertilizers and pesticides to develop organic farming.

INTRODUCTION: India faced several famines in its history, and these famines claimed millions of life. In the famine of 1943, India lost around four million lives in eastern India alone [1]. To solve that situation and to become self-sufficient in food production Government of India launched several scientific ventures. Ultimately in the late 1960s India became self-sufficient in food through green revolution. The success of the green revolution mainly relied on the heavy use of chemical fertilizers, pesticides, wide yielding varieties, and modern mechanical, agricultural instruments [2].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.IJP.6(6).209-15

The article can be accessed online on www.ijpjournal.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.IJP.6(6).209-15

In contrast, modernization of agriculture and dependency on chemical fertilizers and pesticides gradually deteriorates the soil fertility and adversely affects the ecological balance, natural biodiversity, and environment [3]. Adaptation of organic agriculture is the only way to solve this problem [4].

Kunapajala is liquid organic manure mentioned in Vrikshayurveda written by Surapala around 1000 AD and in Lokopakara written by Chavundaraya in 1025 AD. Almost 300 years later, Sarangadhara in his book "Sarangadhara-paddhati" on chapter "Upavanavinoda" also mentioned about Kunapajala [5, 6]. Kunapajala is highly effective for crop plants and can be used as an alternative against chemical fertilizers. A significant development in production was observed due to spraying of Kunapajala in several crop plants including mango (*Mangifera indica*), soapnut (*Sapindus emarginatus*), coconut

(*Cocos nucifera*), kiwi fruit (*Actinidia deliciosa*) and several vegetable crops. Spraying of Kunapajala on tea bushes controlled the attack of tea mosquito bug (*Helopeltis theivora*) and loopers (*Biston suppressaria*) and rat infestation also highly decreased. Narayanan (2006) reported that after spraying Kunapajala rats were disappeared from the tea garden. So it can also be used as an alternative against chemical pesticides and rodenticides [7, 8, 9, 10, 11]. Hence, my motive of this research is to observe the Physical, nutritional and microbial properties of the Kunapajala with different time intervals for determining proper spraying schedule of Kunapajala in crop plants for maximizing its effects on crops.

MATERIALS AND METHODS:

Preparation of Kunapajala:

Ingredient: Bombay duck fish (*Harpedon neherus*, cheap, devoid of scales and easily decomposable) (2.5 kg), powdered sesame oil cake (1 kg), rice husk (1 kg), molasses (1 Kg), Jersey cow urine (7.5 liters).

Procedure: All these ingredients are mixed in an earthen pot, close the container, and allow them to ferment. Stirring twice in a day should be done in both the directions. After 40 days, the solution should be filtered and has to be collected [12].

FIG. 1: KUNAPAJALA- FERMENTATION STATE AND LIQUID EXTRACT AFTER FILTERING

Nutritional and Microbial Analysis of Kunapajala: The physical, nutritional, and biological parameters of Kunapajala were analyzed using scientifically approved standard procedures. The standard procedures performed for the estimations of these parameters are described in **Table 1** and **Table 2**.

TABLE 1: PHYSICAL AND NUTRITIONAL PARAMETERS OF KUNAPAJALA [13, 14, 15]

S. no.	Parameters	Methods	Reference
1	Colour	Visual evaluation	
2	Odor	Sensory evaluation	
3	Mould growth	Visual evaluation	
4	Maggot population	Visual evaluation	
5	pH	pH meter method	Jackson (1973)
6	EC	Conductivity meter method	Jackson (1973)
7	Organic carbon (OC)	Walkley and Black wet digestion	Walkley and Black (1934)
8	Total nitrogen	Microkjeldhal method	Jackson (1973)
9	Total phosphorus	Nitric-Perchloric (9:4) digestion and colorimetry using vanado-molybdo phosphoric yellow color method	Jackson (1973)
10	Total potassium	Nitric-perchloric (9:4) digestion and flame photometry	Jackson (1973)
11	Total calcium	Nitric-perchloric (9:4) digestion and AAS	Jackson (1973)
12	Total magnesium	Nitric-perchloric (9:4) digestion and AAS	Jackson (1973)
13	Total sulfur	Nitric-perchloric (9:4) digestion and Turbidimetry	Massoumi and Cornfield (1963)
14	Total Micronutrients Fe, Mn, Zn, Cu	Nitric-perchloric (9:4) digestion and AAS	Jackson (1973)

TABLE 2: MICROBIAL PARAMETERS OF KUNAPAJALA [16-24]

S. no.	Parameters	Methods	Reference
1	Bacteria	Nutrient agar medium	Atlas and Parks (1993)
2	Fungi	Martin's rose Bengal agar	Martin (1950)
3	*Actinomycetes*	Ken knight's agar medium	Cappuccino and Sheman (1996)
4	PSB	Pikovskaya's mediam	Sundara and Sinha (1963)
5	*Azospirilum*	Nitrogen-free bromothymol blue medium	Dobereiner *et al*. (1976)
6	*Azotobacter*	Jensen's medium	Jensen (1942)
7	*Trichoderma*	Trichoderma specific medium	Saha and Pan (1997)
8	*Pseudomonus*	King's B agar medium	King *et al*., (1954)
9	*Rhizobium*	Yeast extract mannitol agar with congo red	Fred *et al*. (1932)

RESULTS AND DISCUSSION: The color of freshly prepared Kunapajala was brownish orange, and it became darker from the 20 days onwards. As the storage period progressed, the preparation became darker in color without much significant change. Through anaerobic respiration, several gases were produced, and that cause natural liquids and liquefying tissues. They also caused a build-up of pressure combined with the loss of integrity of the skin, and ultimately, the tissue was ruptured. Ruptures in the skin allowed oxygen to re-enter the tissue and provide more surface area for the development of fly larvae and the activity of aerobic microorganisms. For these activities, dark brownish orange color was developed [25, 26].

TABLE 3: MACRO AND MICRO NUTRIENT CONTENT OF KUNAPAJALA ON THE DAY OF PREPARATION, 20 AND 40 DAYS AFTER PREPARATION

	Kunapajala		
Parameters	**On the day of preparation (0 days)**	**20 days after preparation**	**40 days after preparation**
N (ppm)	4690	7238	3486
P (ppm)	208.661	296.260	517.717
K (ppm)	890.396	1589.994	1873.543
Ca (mg/l)	376	452	614
Mg (mg/l)	56	73	88
S (mg/l)	678	857	719
Fe (mg/l)	55	67	72
Zn (mg/l)	6.78	13.63	17.75
Cu (mg/l)	4.76	7.44	8.53
Mn (mg/l)	0.58	1.27	2.06

Fresh preparation of Kunapajala possessed a foul alcoholic smell. The extreme foul odor was observed from 20 to 40 days onwards. The foul alcoholic odor was developed due to putrefaction. Anaerobic metabolism took place, leading to the accumulation of gases, such as hydrogen sulfide, carbon dioxide, methane, cadaverine, putrescine, and nitrogen. The purging of gases and fluids resulted in the strong distinctive odors [26, 27].

Initially, there was no mould growth in Kunapajala whereas it was first observed 5 days after preparation. Mould growth was observed on the liquid surface and also on the sides of the storage vessel from 15 days onwards, the decrease in mould growth was observed in 20 days and was completely absent in 25 days. Fungi consumed energy or food from the decaying tissue and enhanced the decomposition process. Fungi were abundant in the environment. From the air or from any other source they might be appeared in the Kunapajala vessel. But when tissues became totally liquefied or almost decomposed, their population started declining. It was due to the unavailability of food from that decaying tissue. This was the main reason of mould growth in Kunapajala [28, 29, 30].

During decomposition, at initial stages Kunapajala attracted flies and these flies laid eggs on it. From those eggs, maggots were developed. Young maggots spread throughout the container and took food from the decaying tissue. Due to the activity of maggots, the tissue started decomposing faster, and the bacterial activity also highly enhanced. When most of the solid tissue was liquefied, the activity of maggots drastically decreased. This was the reason behind the heavy development of maggots in Kunapajala after 5 days of its

preparation and sudden decline of maggot population after 25 days of its preparation [31-33].

On the day of preparation, Kunapajala showed lowest almost neutral pH (6.74), and after 20 days, it became highly acidic (3.47). Then after 40 days, it became alkaline (8.81). These significant changes highly influence the fungal and bacterial population in Kunapajala. Similar results were also found by Anandan *et al*., (2016), Jani *et al*., (2017) and Ankad *et al*., (2017) [34, 35, 36].

Kunapajala showed highest EC 20 days after preparation (9.72 ds/m), and after that, it started declining (8.57 ds/m, 40 days after preparation). On the day of preparation, it showed the lowest EC (2.55 ds/m). Anandan *et al*.,(2016) and Ankad *et al*.,(2017) [34, 36] also concluded similar trend and results.

Total OC (organic carbon) was highest 40 days after preparation (4.18%), and on the day of preparation, it showed minimum value (1.72%) in Kunapajala. Anandan *et al*. (2016) noticed a similar trend of OC and resulted in his experiment [34].

The highest nitrogen content was recorded 20 days after preparation in Kunapajala (7238 ppm) while 40 days after preparation, it recorded the lowest value (3486 ppm). For the activity of bacteria and maggots, Kunapajala started decomposing faster and due to that N content of the Kunapajala was in an increasing trend. But after 20 days 9-44% of the N was volatized in the form of Ammonia from the solution due to the alkalinity of the Kunapajala solution on that moment [37].

On the day of preparation Kunapajala recorded the lowest value (208.661 ppm) of Phosphorus and 40 days after preparation it recorded the highest value (517.717 ppm) of Phosphorus. Kunapajala contained animal tissue, and animal tissues had high P content. According to Tian *et al*., (1992) [38], organic matters high in P decompose faster and release P significantly, and no volatilization was seen here. So, Kunapajala had an increasing tread of P content during decomposition.

Potassium content was lowest on the day of preparation (890.396 ppm), after that it gradually increased and reached the highest value 40 days after preparation (1873.543 ppm). The activity of fungus and other microorganisms was the reason behind the continuous release of potassium up to 40 days [39]. Highest Ca content was observed 40 days after preparation (614 mg/l), and on the day of preparation, it was the lowest (376 mg/l). Excessive fungus and microbial activity was the reason for the continuous release of Ca up to 40 days [39].

On the day of preparation Mg content was the lowest (56 mg/l) and after 40 days, Mg content recorded the highest value (88 mg/l). Fungal and microbial activity was the main cause behind the gradual release of Mg in Kunapajala [39]. S content was lowest on the day of preparation (678 mg/l), and after 20 days it recorded the highest value (857 mg/l), but then S content started declining. Due to the excessive volatile release of hydrogen sulfide, after 20 days S content started declining [39].

Highest Fe content was recorded 40 days after preparation (72 mg/l), and on the day of preparation, it was the lowest (55 mg/l). Due to fungal and bacterial activity, gradual release of Fe was noticed in Kunapajala [40].

On the day of preparation, Zn content was minimum (6.78 mg/l) and 40 days after preparation, it became maximum (17.75 mg/l). Gradual increase of Zinc content was noticed in Kunapajala due to the activity of fungus and bacteria [41, 42, 43]. Cu content was maximum 40 days after preparation (8.53 mg/l), and on the day of preparation, it recorded the lowest value (4.76 mg/l). Continuously increasing trend of Cu content was observed due to the activity of several fungal and bacterial species [41, 42]. Highest Mn content was noticed 40 days after preparation (2.06 mg/l), and on the day of preparation, the Mn content recorded the lowest value (0.58 mg/l). Due to heavy microbial interaction or activity inside Kunapajala might be the reason behind this trend.

Fungi population was highest 40 days after preparation (33×10^8 CFU/ml), and it was lowest on the day of preparation (4×10^4 CFU/ml). This gradual increasing trend was noticed due to enhanced activity of early stage fungi ascomycetes, deuteromycetes and saprophytic basidiomycetes and late-stage fungi ectomycorrhizal basidiomycetes in Kunapajala with time [43].

On the day of preparation, Kunapajala recorded lowest *Actinomycetes* population (3×10^3 CFU/ml). After that, it increased continuously and reached the highest 40 days after preparation (5×10^8 CFU/ml). Continuous decomposition of a complex mixture of polymers in dead animal tissues was the prime reason for continuous development of *Actinomycetes* population in Kunapajala [44, 45, 46].

TABLE 4: BENEFICIAL MICROBIAL POPULATION OF KUNAPAJALA ON THE DAY OF PREPARATION, 20 AND 40 DAYS AFTER PREPARATION

	Kunapajala		
Parameters	**On the day of preparation (0 days)**	**20 days after preparation**	**40 days after preparation**
Fungi (cfu/ml)	4×10^4	16×10^7	33×10^8
Actinomycetes (cfu/ml)	3×10^3	6×10^4	5×10^8
Pseudomonas (cfu/ml)	5×10^3	8×10^{10}	13×10^{10}
PSB(cfu/ml)	2×10^5	15×10^{10}	21×10^{10}
Azotobacter (cfu/ml)	7×10^4	9×10^{12}	13×10^{12}
Azospirilum (cfu/ml)	11×10^3	8×10^8	13×10^{10}
Rhizobium (cfu/ml)	2×10^3	6×10^6	4×10^{11}
Trichoderma (cfu/ml)	6×10^3	18×10^8	21×10^8

The highest population of *Pseudomonas* was noticed 40 days after preparation (13×10^{10} CFU/ml) in Kunapajala, and on the day of preparation it recorded the lowest (5×10^3 CFU/ml). This type of increasing trend up to 40 days in Kunapajala was also concluded by Ali *et al*., (2012) [47]. PSB population was highest on the day of preparation (2×10^5 CFU/ml), and it became a maximum 40 days after preparation (21×10^{10} CFU/ml) in Kunapajala. A similar trend of population growth was also observed by Ali *et al*. (2012) [47] in Kunapajala.

On the day of preparation, *Azotobacter* population had the lowest value (7×10^4 CFU/ml) in Kunapajala and after 40 days it became the highest (13×10^{12} CFU/ml). Presence of *Azotobacter* in Kunapajala and this type of growth trend was justified by Ali *et al*., (2012). Highest *Azospirilum* population was noticed 40 days after preparation (13×10^{10} CFU/ml), and on the day of preparation, the lowest value was found (11×10^3 CFU/ml). Ali *et al*., (2012) approved the existence of *Azospirilum* in Kunapajala and its growth behavior in it [47].

Lowest *Rhizobium* population was found on the day of preparation (2×10^3 CFU/ml), and after 40 days highest *Rhizobium* population (4×10^{11} CFU/ml) was noticed in Kunapajala. Ali *et al*., (2012) also concluded a similar trend of population growth of *Rhizobium* in Kunapajala [47]. *Trichoderma* population was highest 40 days after preparation (21×10^8 CFU/ml) in Kunapajala and on the day of preparation it had the lowest population (6×10^3 CFU/ml). *Trichoderma* had significant contribution in decomposition and biodegradation of organic matters and due to that, the population of *Trichoderma* in Kunapajala had a continuous increasing trend up to 40 days [48].

CONCLUSION: The study concludes that Kunapajala has great potential as organic manure because of its high nutrient content and beneficial microbial population. It can also be used as an organic pesticide because most of the fungus and bacteria present in it have good bio-control properties. From the study, it is clear that the nutrient content and microbial population in Kunapajala is continuously varied with time.

So, foliar and soil application of Kunapajala from the beginning of its preparation and up to 40 days of its preparation will be helpful for the crop and soil because we can utilize its total potential. The ingredients required to prepare it is easily available and cheap comparing with chemical fertilizers and pesticides.

On the other hand, they are easily available also. So the use of Kunapajala instead of chemical fertilizer and pesticide is highly useful to increase the crop yield and to maintain the productivity of the soil. Moreover, it is highly cost-effective for farmers.

ACKNOWLEDGEMENT: My special thanks to Saddam da, Salim da, Amar da and my classmate Hiralal Mandi and Somesa Ghosh for their cooperation during laboratory work.

CONFLICT OF INTEREST: Nil

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How to cite this article:
Chakraborty B, Sarkar I, Maitra S, Khan AM, Bandyopadhyay S and Sinha AK: Nutritional and microbial analytical study of Vedic liquid organic manure cum pesticide Kunapajala with different storage time interval. Int J Pharmacognosy 2019; 6(6): 209-15. doi link: http://dx.doi.org/10.13040/IJPSR.0975-8232.IJP.6(6).209-15.