Original Research Article

PRODUCTION OF KUNAPAJALA, ITS NUTRITIONAL CONTRIBUTIONS, MICROBIAL AND PESTICIDE EFFECT

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Abstract

A study on nutritional and microbial analysis of Kunapajala with different storage time interval was conducted in the Department of Soil Science & Agricultural Chemistry and the Department of Plant Pathology, UBKV, Coochbehar-736165, West Bengal during March, 2019. ~~Motive~~ The motive of this work was to estimate the ~~physico-chemical~~physicochemical properties, macro and micro nutrient content and various microbial load of Kunapajala with different storage time interval. Kunapajala had the highest P, K, Ca, Mg, Fe, Zn, Cu & Mn 40 days after preparation, and the ~~it had~~ highest N and S 20 days after preparation. It had the highest beneficial microbial load of Fungi, *Actinomycetes*, *Pseudomonus*, Phosphorus Solubilising Bacteria (PSB), *Azotobacter*, *Azospirillum*, *Rhizobium* and *Trichoderma* 40 days after preparation. So, continuous foliar and soil application of Kunapajala from 20 days after preparation to 40 days after preparation was beneficial to get maximum utilization. Moreover, Kunapajala can be used as an alternative against chemical fertilizers and pesticides to develop organic farming.

Key words: Kunapajala, Liquid organic manure, ~~organic~~ Organic farming, Organic pesticide, ~~organic~~ Organic fertilizer.

1. Introduction

India faced several famines in its history and these famines claimed millions of ~~life~~lives. In the famine of 1943, India lost around four million lives in eastern India alone (Dyson and Maharatna 1991).To solve that situation and to become self-sufficient in food production, ~~Govt.~~the government of ~~Of~~ India launched several scientific ventures. Ultimately in late 1960~~'~~s, India became self-sufficient in food through green revolution. The success of green revolution mainly relied on the heavy use of chemical fertilizers, pesticides, high yielding varieties and modern mechanical agricultural instruments (FAO, 2009). 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 (Paull, 2011). Adaptation of organic agriculture is the only way to solve this problem (Manna *et al*., 2005). The procedure of preparation of Kunapajala was mentioned in ~~Vrikshaurveda~~ Vrikshayurveda written by Surpala. According to verse 101, 102, 103 and 104 of ~~Vrikshaurveda~~ Vrikshayurveda, it could be prepared by mixing excreta, marrow of the bones, flesh, brain and blood of the boar with water. After that the mixture should be boiled and stored in an iron pot after adding sufficient quantity of husk, sesame oil cake, honey, black gram and ghee. As per availability, the blood, flesh and marrow of fish, goat or other animals could be used for the preparation. The items should be taken at random, no specific proportion is mentioned. Verse 106 of ~~Vrikshaurveda~~Vrikshayurveda explained that Kunapajala was highly effective for the crop plants. A significant increase 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 bringal (*Solanum melongana*). Spraying of Kunapajala on tea bushes controlled the attack of tea mosquito bug (*Helopeltis theivora*) and loopers (*Biston suppressaria*). Narayanan (2006) reported that after spraying Kunapajala (made of rat flesh, ~~Musika~~ Mushika kunapa), rats were totally disappeared from tea garden. So it can also be used as an alternative against chemical pesticides and rodenticides (~~Ayangara~~Ayangarya, 2004a, 2004b, 2005, 2006a, 2006b) (Narayanan, 2006) (Bhat and Vasanthi 2008). Hence, my motive of this research is to observe the ~~Physical~~ physical, nutritional and microbial properties of the Kunapajala with different storage time intervals. Due to several microbial interactions, the nutritional status of Kunapajala is continuously changing. By ~~Studying~~ studying the nutritional content and microbial population ~~with different time interval~~, we can understand the potentiality of Kunapajala in different time intervals. According to that, proper spraying schedule of Kunapajala should be recommended to the farmers for maximising crop yield.

2. Materials and Methodology

2.1. Preparation of Kunapajala

Ingredient: Bombay Duck fish [(*Harpedon nehereus ~~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 litres).

Procedure: All these ingredients were mixed in an earthen pot, closed the container and allowed them to ferment. Stirring twice in a day should be done in both ~~the~~ directions. After 40 days the solution should be filtered and ~~had to be~~ collected (Sarkar *et al*., 2014).

Kunapajala- Fermentation State and liquid extract after filtering

2.2. Nutritional and microbial analysis of Kunapajala

The physical, nutritional and biological parameters of Kunapajala were analysed on the day of preparation (0 days), 20 days after preparation and 40 days after preparation 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 chemical properties of Kunapajala

Sl. No.	Parameters	Methods	Reference
1	Colour	Visual evaluation	
2	Odour	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	Micro kjeldhal method	Jackson (1973)
9	Total Phosphorus	Nitric-Perchloric(9:4) digestion and colorimetry using vanado-molybdo phosphoric yellow colour 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 Sulphur	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. Biological properties of Kunapajala

Sl. 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 medium	Sundararao ~~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)

3. Results and Discussion

Table-3. Physical and Physico-chemical parameters of Kunapajala

KUNAPAJALA			
Parameters	**On the day of preparation (0 days)**	**20 days after preparation**	**40 days after preparation**
Colour	Light brownish orange	Brownish orange	Dark brownish orange
Odour	Mild alcoholic smell	Foul alcoholic smell	Extreme foul alcoholic smell
Mould growth	No mould growth	Heavy mould growth	No mould growth
Maggot Population	No maggot found	Heavy maggot growth	No maggot found
pH	6.74	3.47	8.81
EC (ds/m)	2.55	9.72	8.57
Total OC (%)	1.72	2.55	4.18

The colour of freshly prepared Kunapajala was brownish orange ~~and~~ then it became darker from the 20th days onwards. As the storage period progressed, the preparation became darker in colour without much significant change. Through anaerobic respiration, several gases were produced and that cause natural liquids and liquefying tissues. They also caused 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 colour was developed (Janaway *et al.*, 2009; Carter *et al.*, 2008).

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Fresh preparation of Kunapajala possessed a foul alcoholic smell. Extreme foul odour was observed from 20 to 40 days onwards. Foul alcoholic odour was developed due to putrefaction. Anaerobic metabolism took place, leading to the accumulation of gases, such as hydrogen sulphide, carbon dioxide, methane, cadaverine, putrescine and nitrogen. The purging of gases and fluids resulted the strong distinctive odours (Carter *et al.*, 2008; Payne, 1965).

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 the 15th days onwards, the decrease in mould growth was observed in the

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20th days and was completely absent in the 25th ~~days~~. Fungi consumed energy or food from the decaying tissue and enhanced the decomposition process. Fungi were abundant in the environment. ~~From air or from~~Through air or any other source they might be appeared in the Kunapajala vessel~~. ,~~ ~~But~~ but when tissues became totally liquefied or almost decomposed, their population started declining. It was due to unavailability of food from that decaying tissue (Hawksworth and Wilthshire, ~~*et al*.,~~ 2011) (Schwarz *et al*., 2015) (Hitosugi *et al*., 2006).

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During decomposition, at initial stages, Kunapajala attracted flies ~~and these flies~~which 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 enhanced. This was the reason behind the ~~heavy~~ high development of maggots in Kunapajala after 5 days of its preparation. After 25 days of its preparation due to the loss of readily available cadaveric material, maggot population drastically reduced (Anderson, 2000), (Fuller, 1934) (Morovic-Bodac, 1965), (Carter and Tibbett, 2008), (Janaway *et al*., 2009)

On the day of preparation, Kunapajala showed ~~of~~ pH (6.74) and after 20 days it became highly acidic in nature (3.47). Then after 40 days it became alkaline in nature (8.81). Animal tissue decomposition initially created an alkaline environment and due to microbial activity it became acidic after 20 days. When decomposition was totally completed, it became alkaline again (Carter, 2005; Hopkins *et al*., 2000; Rodriguez and Bass 1985) (Gill-King, 1997; Towne, 2000). Similar results were also found by Anandan *et al*., (2016), Jani *et al*., (2017) and Ankad *et al*., (2017) in Kunapajala.

Kunapajala showed highest EC 20 days after preparation (9.72 ds/m) due to high acidic nature of the solution 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) (Carter, 2005; Hopkins *et al*., 2000; Rodriguez and Bass 1985) (Gill-King, 1997; Towne, 2000). Anandan *et al*., (2016) and Ankad *et al*., (2017) 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. In decomposition, physical breakdown and biochemical transformation of complex organic molecules occurred, due to that, several organic carbon compounds were synthesized (Juma, 1998). This was the reason for continuous increase of OC in Kunapajala. Anandan *et al*., (2016) noticed similar trend of OC and results in his experiment.

Physical and physicochemical ~~physic-chemical~~ parameters of Kunapajala were mentioned in Table-3.

The highest N content was recorded 20 days after preparation in Kunapajala (7238 mg/dm^3), while on the day of preparation it recorded the lowest value (3486 mg/dm^3). 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~~ but after 20 days, 9-44% of the N was volatized in the form of Ammonia from the solution due to alkalinity of the Kunapajala solution ~~on~~ at that moment (Kirchmann and Witter 1989). Ankad *et al*., (2017) and Jani *et al*., (2017) also concluded similar trend and results in their experiment.

On the day of preparation, Kunapajala recorded the lowest value (208.661 mg/dm^3) of P, while~~and~~ after 40 days ~~after~~ of preparation it recorded the highest value (517.717 mg/dm^3)~~ of P~~. Kunapajala contained animal tissue ~~and animal tissues~~which had high P content. According to Tian *et al*., (~~1992~~1995), organic matters high in P decompose faster and release P significantly. So, Kunapajala had increasing trend~~tread~~ of P content during decomposition. Ankad *et al*., (2017) and Jani *et al*., (2017) also analysed the P content of Kunapajala and found similar results.

K content was lowest on the day of preparation (890.396 mg/dm^3), after that it was gradually increased and reached the highest value~~ at~~ 40 days after preparation (1873.543 mg/dm^3). Activity of ~~fungus~~ fungi and other microorganisms was the reason behind continuous release of K up to 40 days (Carter *et al*., 2007).

The ~~Highest~~ highest Ca content was observed 40 days after preparation (614 mg/l), ~~and~~ while on the day of preparation it was the lowest (376 mg/l). Excessive fung~~ius~~i and microbial activity was the reason for continuous release of Ca up to 40 days (Carter *et al*., 2007).

On the day of preparation, Mg content was the lowest (56 mg/l) ~~and~~whereas after 40 days, ~~Mg content~~it recorded the highest value (88 mg/l). Fungal and microbial activity was the main cause behind gradual release of Mg in Kunapajala (Carter *et al*., 2007).

S content was the lowest ~~on the~~ on the day of preparation (678 mg/l), ~~and~~ whilst after 20 days it recorded the highest value (857 mg/l),~~ but~~ then S content started declining. Due to excessive volatile release of hydrogen sulphide, after 20 days S content started declining (Carter *et al*., 2007).

The ~~Highest~~ highest Fe content was recorded 40 days after preparation (72 mg/l), while ~~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 (Dent *et al*., 2004).

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 activity of ~~fungus~~ fungi and bacteria (Hodson *et al*., 2001, Kearney *et al*., 2000 and Deydier *et al*., ~~2003~~2005).

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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 activity of several fungal and bacterial species (Hodson *et al*., 2001, Kearney *et al*., 2000 and Deydier *et al*., ~~2003~~2005).

The ~~Highest~~ 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). Heavy microbial interaction or activity inside Kunapajala might be the reason ~~behind~~ of this trend and result.

The macro and micro nutrient content of Kunapajala was mentioned in Table-4.

Table-4. Macro and micro nutrient content of Kunapajala

KUNAPAJALA			
Parameters	**On the day of preparation (0 days)**	**20 days after preparation**	**40 days after preparation**
N mg/dm^3	3486	7238	4690
P mg/dm^3	208.661	296.260	517.717
K mg/dm^3	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

Fungi population was the highest 40 days after preparation (33×10^8 cfu/ml) and it was the 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 (Carter et al.and Tibbett, 2003).

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On the day of preparation, Kunapajala recorded the lowest *Actinomycetes* population (3×10^3 cfu/ml). After that it increased continuously and reached the highest at 40 days after preparation (5×10^8 cfu/ml). Continuous decomposition of complex mixture of polymers in dead animal tissues was the prime reason for continuous development of *Actinomycetes* population in Kunapajala (Goodfellow and Williams 1983, McCarthy and Williams 1992, Stach and Bull 2005).

The highest population of *Pseudomonus* was noticed 40 days after preparation (13×10^{10} cfu/ml) in Kunapajala, while 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).

PSB population was the highest on the day of preparation (2×10^5 cfu/ml) and then it became maximum at 40 days after preparation (21×10^{10} cfu/ml) in Kunapajala. Similar trend of population growth was also observed by Ali *et al*., (2012) in Kunapajala.

On the day of preparation, *Azotobacter* population had the lowest value (7×10^4 cfu/ml) in Kunapajala and while 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).

The Highest 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 and growth behaviour of *Azospirilum* in Kunapajala ~~and its growth behaviour in it~~.

The ~~Lowest~~ lowest *Rhizobium* Population was found on the day of preparation (2 x 10^3 cfu/ml) and after 40 days, the highest Rhizobium population (4 x 10^{11} cfu/ml) was noticed in Kunapajala. Ali *et al*., (2012) also concluded similar trend of population growth of *Rhizobium* in Kunapajala.

Trichoderma population was highest 40 days after preparation (21 x 10^8 cfu/ml) in Kunapajala and on the day of preparation it had the lowest population (6 x 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 ~~an~~ a continuous increasing trend up to 40 days (Woo *et al*., ~~2004~~2014).

Microbial population of Kunapajala was mentioned in Table-5.

Table-5. Microbial population of Kunapajala

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

4. Conclusion

The study concludes that Kunapajala has high nutrient content and beneficial microbial population. Nutrient content of Kunapajala is highly influenced by its microbial population. Fungi will help to breakdown complex organic compounds and produce simple organic and inorganic compounds useful for plants. *Azotobacter*, *Azospirilum* and *Rhizobium* help to fix more N in crop field. PSB enhance the P solubilisation in crop field. *Actinomycetes* help to decompose complex organic molecules and antagonistic potential of *Pseudomonus*, ~~and~~ while *Trichoderma* will help to protect the crop from soil-borne diseases. Microbial population is continuously increasing and ~~it become~~ became the highest after 40 days. So, application of Kunapajala after 40 days is beneficial for crops~~.~~, ~~But~~ but N and S content of Kunapajala is the highest 20 days after preparation~~.~~, ~~So~~ so to exploit that, spraying of Kunapajala after 20 days is also recommended. Spraying of Kunapajala on the day of preparation is not recommended because ~~on that day~~the microbial population and nutrient content ~~was~~ is the minimum and most of the organic matter is not properly decomposed, so they will not be

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highly available for the cop plants. So foliar and soil application of Kunapajala from 20 days of its preparation to 40 days of its preparation is recommended for the crop and soil because we can utilise its total potential. The ingredients required to prepare it ~~is~~ are easily available and cheap comparing with chemical fertilizers and pesticides. The crops produced using Kunapajala will be free from any harmful chemical residues. So, it is healthy for the consumer. Moreover, use of Kunapajala instead of chemical fertilizer and pesticide is highly useful to increase the crop yield, soil productivity and farmer's income.

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Comment [A5]: The second reference is from google scholar. Which one is correct? Then correct it in the text

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Comment [A6]: This reference is not in the text. Add to the text or delete the reference

Comment [A7]: Find the previous comment A6

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