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Advances in Bioresearch

ORIGINAL ARTICLE

Bio formulation of KKKP and its Physicochemical properties for Sustainable soil Fertility

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ABSTRACT
In this study liquid organic preparations of KKKP is prepared with 4 different bases and its physochemical properties like pH, Electrical conductivity, viscosity, solubility, vapour pressure, water partition co efficient, Carbon and nitrogen were evaluated. Base 1 is mixture of cow product have EC 4.2 ds m^{-1} and pH 8.6 is prepared and mixed with the base ii consist of fruit waste and sprouts with PH 7.4 and EC 3.2 ds m^{-1}. Bases iii enriched fermented product of fish meal and jaggery having EC 4.4 ds m^{-1} and pH 8.3 is added to the above mixture. Along with this three preparation final product is obtained by mixing base iv component prepared by using red algal extract and selected medicinal plants like Vitextrifola, D.metal, C .gigantea, ginger, garlic were taken and grinded well and mixed with the above bases. The phytochemical analysis reveals presence of important phytochemicals like alkaloid, flavonoids, phenols, tannins, coumarins, phlobatanins and saponins were detected.Heavy metals such asArsenic (As), Lead(Pb),Chromium and Nickelin the final KKKPis noted as permissible level 0.322≥0.12l≥0.1≥0.08 mg/L. Arsenic (As) and Cadmium(Cd) were not detected.
KEYWORD:*KKKP, Vitex trifola, Fermented Product, Medicinal Plants and Heavy Metals.*

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INTRODUCTION

Increasing world's population is demands high global crop cultivation needs to double the productivity. Factors of elevated food demands and diminishing agricultural land stressed the strategies to strengthen the agricultural systems without affect the environment [1]. Subsequently agricultural production must increase by 50% to overcome worldwide demands for food. Many ecological factors determine the quality fruit size, yield, resistance, colour, shape, firmness (organoleptic) and nutrient content [2]. Organic farming was well developed between 2000 to 2013 and plays most important part in modern agriculture system. Organic agriculture is a beneficial agro-system that preserves soil quality by maintaining soil fertility, storing carbon dioxide, reducing fossil fuels, preserving landscapes and preserving biodiversity [3]. Pest control in organic farming is a well-developed and commonly used approach to the conservation of ecological processes and biodiversity.

Microbes and plant coupled formulations also known as bioformulations are more robust than synthetic chemicals. A bioformulation become effective when it does not affected by Conditions of the field, customer base and consistency and cost-effectiveness [4]. Bioformulation enhances the durability of antibiotics, enzymes, phytohormones, siderophores and compounds in contact with plant [5]. Organic farming enhances nutrient availability and thus overcome lower yield horticulture [6].Liquid biofertilizers have become popular due to 15 % enhanced additional yields in their crops. The demand for liquid biofertilizers by the farmers has increased as they act as biostimulant. Bio stimulants are materials stimulate and promote plant growth with micronutrient. Biostimulants may contain micro level of plant hormones and other compounds but if the registered as a plant progress supervisors. Du Jardin stated that plant biostimulants (PBs) were supplied to plants primarily with the goal of improving nutritional

uptake efficiency, but also increasing abiotic stress tolerance and/or crop quality characteristics, regardless of their nutrient content [7]. The sources of sea weed extract, protein hydro-lysates, humic and fulvic acids, silicon, chitosan, inorganic compounds and beneficial fungi and bacteria are preominently used and found to be potent biostimulants[8].

Organic preparations used to prevent the growth of microorganisms such as fungi or bacteria can be made from a variety of natural phytochemical sources includes plant extracts, humic acid and polysaccharides along with variety of antagonsistic bacteria or fungi also used asbiocontrol of fruit pathogens [9].Advanced methods of bio formulations preparations are under intense research, KKKP is the safe and effective bio formulation which performs three in one action like, Promotes plant growth and increases the crop yield, Pest control, Fungal and bacterial diseases in various crops.

MATERIAL AND METHODS

Study materials

Chemicals and media: Chemicals, microbial culture media and ingredients used for the development of different bioformulations were of laboratory chemical grade and were purchased from the HI-media market. The culture media used in this study included Nutrient agar (NA), King's B (KB), SDA Sabourd dextrose agar and Potato Dextrose Agar (PDA). Brinjal, laddys finger, chilli seeds were obtained from seeds of Andavan arts and science college microbiology department.

BIOFORMULATION OF KKKP

The quantity and components of KKKP was given in table 2. A mixture of five components such as cow dung, cow urine, milk, curd, ghee, were prepared and used as base I fermented alone for 30 days. Base 2 is prepared with mixture of *Banana, Manilkara zapota, Carica papaya, Cucurbita pepo*, Coconut water, Sugar cane juice, Ground nut cake and Sprouted Nawadhanya paste and fermented for 30 days. Base 3 prepared by mixing equal volume of jaggery and fish waste and further incubated for 3 months with subsequent mixing. Base 4 contain extract of herbal plants such as *V. trifolia, Datura metel, Calotrips gigantia,* rhizome of *Zingiber officinale*, *Allium sativum,* red algae were grinded well and incubated under dark anaerobic condition at room temperature for 30 days. Twice a day, the all the mixture is stirred for 20 min with a wooden stick daily as described above for 20 min to facilitate aeration. End of incubation day physicochemical properties of bases were tested as described in 3.4 and 3.5.The end product KKKP is prepared by mixing of all four bases in a sterile plastic barrel and used for further studies.

Preparation poly herbal extract

V. trifolia, Datura metel, Calotrips gigantia, Aloe vera were collected from Karur *district,* Tamil Nadu, India. All parts of the plants were brought to the laboratory, washed twice in tap water to remove the soil particles and dried before extraction procedure. The dried leaves of C*alotrips gigantia* and *V. trifolia*, the whole plant of *Datura metel*, rhizome of ginger and garlic bulbs were used for this study. Each 500 g of each crushed material was placed separately in a columnar extraction system and 20L of water and fermented for 10 days. The extraction process was carried out using simple filtration.

Preparation poly algal extract

Marine red algae (*Gracilaria*sp) were collected from mandapam region, Rameshwaram in a sterile polythene bags and brought to lab under ice cold condition. The surface soil removed by washing under distilled water until removal of soil and other debris then dried in shadow open air for 24 h. About 5 kg of red algae grinded well using mixture grinder and the content was diluted up to 5 L.

Physico-chemical properties of KKKP

pH

The pH of fermented KKKP was tested using digital pH meter after calibration with suitable buffer. The solid particles removed by filtration and aqueous phase were tested followed by storage at different temperature.

Electrical conductivity

KKKP X-formula and Rocket 44 EC formula and KKKP base 1-4 samples electrical conductivity tests were carried out by using Eutech Instruments PCD 650. Raw and Diluted samples was prepared and Multiparameter meter at room temperature under argon atmosphere. A custom made sample holder was used to seal the conductivity probe in place and to provide similar experimental conditions. The meter was calibrated previously with standard KCl solutions with different mass fractions. The values of conductivity measured using the measured resistance was corrected to 20 °C with co-efficient 0.020 °C

Viscosity

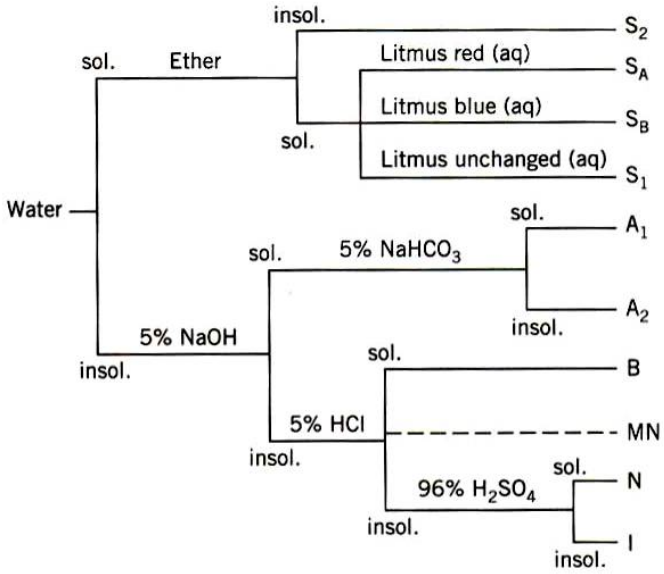
The viscometer was calibrated Brookfield DV-E viscometer with a 3.2 mm diameter spindle and a 9 mm inside diameter tube that was set in water jacket with temperature controlled to ±0.02 °C. This was calibrated using Cannon N1000 calibration oil to within ±1% over the rotational speed range of the

viscometer. KKKP base of KKKP, CVD, DM were subjected for this test. The calibrated electrode is dipped inside the sample and readings were recorded.

Solubility test

The solubility of KKKP among Water, 5% NaOH, 5% $NaHCO_3$, 5% HCL, Cold concentrate of H_2SO_4 was used. About 1 part of KKKP is mixed with 4 part of water and mixed well and the solubility is recorded. The test is further continued with acid and base medium and the solubility is recorded.

FIGURE-1.SCHEMATIC REPRESENTATION OF SOLUBITY TEST



Water partition coefficient of KKKP

Octanol was first saturated with water, for this one litre of MilliQ water was taken and saturated with 250 ml of 1-octanol for 24h at 25 °C. To this the test compound are equilibrated under stirred reactor at fixed temperature. The solubility of the test compounds in *n*-octanol was determined by slow-stirring method which reduces turbulence and thereby enhances the exchange between n-octanol and water without microdroplets formation. The lower water phase is collected from the bottom of the vessel, whereas the n-octanol phase is sampled using a syringe and the undisturbed the boundary layer collected by separating funnel. The solubility of compounds in n-octanol was determined by UV spectrophotometer (nm).

$K_{ow=OD}$ in n Octanol/ OD in water phase

Polarity test

One part of KKKP formulation mixed with four part of Methanol, Acetone, Hexane, Tolune, and Benzene and vigoursly mixed then centrifuged at 5000 rpm. The formation of precipitation at bottom of tube indicates insoluble nature.

Vapour Pressure

The volume of sample container is 50 ml. It was 80% filled with the sample. A bath capable of maintaining a constant temperature setting between ambient temperature and 120°F.A gauge with a range of 0 to 5 psi capable of being read to 0.025 psi. After the assembled vapour pressure apparatus has been immersed in the bath for at least 5 minutes, tap the pressure gage lightly and observe the reading.

PHYTO CHEMICAL STUDY

QUALITATIVE PHYTOCHEMICAL ANALYSIS

Test for coumarin

The 3 ml of 10% NaOH was added to 2 ml of extract formation of yellow colour indicates the presence of coumarin.

Test for terpenoids (SALKOWSKI TEST)

To 3 mL of the extract, 2 ml of chloroform was added and finally carefully treated with 1 mL H_2So_4. Production of dark reddish brown interface considered aspresence of terpinoids.

Test for flavonoids (ALKALINE REAGENT TEST)

About 3 mL of dilute ammonia, 2 mL plant extract and 1 mL concentrated sulphuric acid (H_2SO_4) was mixed slowly in a test tube. Yellow coloration in each extract showed the presence of flavonoids.

Test for saponins
The extract was diluted with 20ml of distilled water and it was agitated in a graduated cylinder for 15 minutes. The formation of 1 cm layer of foam shows the presence of saponins. The extract, 1ml was treated with 1% lead acetate solution. Formation of white precipitate indicates the presence of saponins.
Test for Steroids
2 ml of chloroform and concentrated H_2SO_4 were added with the 5 ml aqueous plant crude extract. Formation of red color at the chloroform layer indicate the presence of steroids.
Test for carbohydrate
To 2 ml of barfoed's reagent 0.5 mL plant extract was added and tube is kept under boiling bath for 5 min. formation of Reddish or yellow precipitate confirms test is positive on presence of carbohydrate.
Test for tannins
Few drops of 0.1% ferric chloride were added to diluted plant extract and observed formation of brownish green or blue-black coloration indicative of the presence of tannins.
Test for alkaloid Mayer s test:
Extracts were dissolved individually in dilute hydrochloric acid and filtered. The filtrates were used to test the presence of alkaloids. Filtrates were treated with Mayer s reagent. Formation of a yellow cream precipitate indicates the presence of alkaloids.
Test for lignin
Take 2 ml of extract in a test tube and add few drops safranin solution mix well and allow it stand for 5 min. formation of pink colour indicates the presence of lignin.
Test for proteins
To 1 ml of the extract, 2ml of alkaline $CuSo_4$ reagent and 0.5 mL follins reagent were added and kept at RT for 30 min formation of blue color indicate presence of protein.
Detection of Phenols:
Ferric chloride test: 5 ml extracts were treated with few drops of ferric chloride solution. Formation of bluish black colour indicates that the presence of phenol.
Phlobatannins
An aqueous extract of the plant sample was boiled with 1% aqueous HCL. Deposition of red precipitate was taken as an evidence for the presence of phlobatannins
Quantitative Phytochemical Analysis (Base 4)
1. Estimation of Alkaloids:
5 ml phosphate Buffer (pH 4.7) was added and 5 ml BCG solution and 4 ml of chloroform was taken and added to 1 mL sample and mixed vigorously. The absorbance of the compound present in chloroform was measured at 470 nm against blank and plotted on standard Atropineequivalents.
Estimation of flavanoids:
Total flavonoid content was determined by Aluminium chloride method using catechin as a standard. 1ml of test sample and 4 ml of water were added to a volumetric flask (10 ml volume). After 5 min 0.3 ml of 5 % Sodium nitrite, and10% Aluminium chloride was added and incubatied at room temperature. After 5 min 2 ml of 1 M Sodium hydroxide was added to the reaction mixture. Immediately the final volume was made up to 10 ml with distilled water. The absorbance of the reaction mixture was measured at 510 nm against a blank spectrophotometrically. Results were expressed as catechin equivalents (mg catechin/g dried extract).
Estimation of Saponins:
Plant extract was dissolved in 80% methanol, 2ml of ethanolic solution of Vanilin and the 2 ml of 70 % sulphuric acid solution was added, mixed well and heated on a water bath at 60^{o}c for 10 min. The absorbance was measured at 544 nm against reagent blank. Diosgeninis used as a standard material and compared the assay with Diosgenin equivalents.
Estimation of Steroids:
1ml of plant extract, 2ml of each Sulphuric acid (4N,) and iron (III) chloride (0.5% w/v), were mixed well and then 0.5 ml of potassium hexacyanoferrate (III) solution (0.5% w/v,) was added. The mixture was heated in a water-bath at 70^{o} C for 30 minutes with occasional shaking and diluted to the mark with distilled water. The absorbance was measured at 780 nm against the reagent blank.
Total Phenolic Content (TPC):
Determination of Total Polyphenols (TP) For the optimization and standardization of the spectrophotometric method using the Folin-Ciocalteau reagent, three parameters were analyzed: The total phenolic content (TPC) of the different plant extracts was determined using Folin–Ciocalteu reagent, with slight modification. In brief, 1 mL of crude extract (1 mg/mL) was mixed with 1 mL of Folin–Ciocalteu reagent followed by the addition of 5 mL of distilled water in a volumetric flask. After 5 min,

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1 mL of sodium carbonate (10%) was added and shaken vigorously. Then, the final mixture was incubated in the dark condition at room temperature for 60 min, and the absorbance was measured against the blank at 370 nm using a UV-VIS spectrophotometer. The total phenolic content of plant samples was calculated from the calibration curve of standard gallic acid (10-250 mg/L) and expressed as mg gallic acid equivalent (GAE) per gram dry extract weight.

Heavy metal Analysis of KKKP:

Determination of heavy metal in waste and soil samples: Cr, Ni, Ar, Pb, Cd, Hg were analyzed from the wastes and soil sample. Analyses were done at the soil science division laboratory, Tiruchirappalli. For the determination of total metal concentration exactly 1 gm KKKP is mixed with 9 mL of HNO_3: HCl (1:3). Then the content was evaporated to dry and again 5 mL of aquaregia was added. This process was repeated 2–3 times for efficient extraction of metals. Then the digest was filtered through a filter paper (Whatman no. 42). The determination of different heavy metals from waste samples was done by atomic Absorption Spectrophotometer (AAS) (PG990, England). A standard curve was prepared by plotting the absorbance reading on. Y–axis versus the concentration of each standard solution of metal on X–axis. Then the concentration of metals was calculated in the sample by plotting the AAS reading on the standard curve.

RESULT AND DISCUSSION

Physiochemical properties of KKKP

The effect of KKKP formulation on nutrient value, germination, growth, yield and pest control on vegetable crops okra, chilli and brinjal were evaluated. The bio formulation of KKKP using mixture bases ofpanchakavya mix, jaggery-fishmeal, and Poly herbal and red algal extract mixed and fermented for end product KKKP. Experimental data of physiochemical nature such as pH, Viscosity, Eletrical conductivity(EC), polarity, water partition coefficient of each base of KKKP was estimated and values are given in table 1. The pH of base I-IV are varies ranges from 6 to 8. The pH of KKKP at RT is recorded as 3.5. The pH under various temperatures is given in figure 1. The end product have pH maximum of 3.5 between 10-30° C and there is no changes during raising of temperature between 40-100° C. the pH of chemical pesticide was 6.Pathak and Ram also found low pH in Panchagavyadue to production of several organic acids in it during fermentation. Alcohol (methanol, propanol, butanol and ethanol) production in Jeevumrutha as a by-product of fermentation made it alkaline in nature [10-11].Sasyamrutha was alkaline in nature might be due to release of carbon dioxide and other volatile metabolites like isothiocyanate, CNS, nitriles and other degradation products [12].

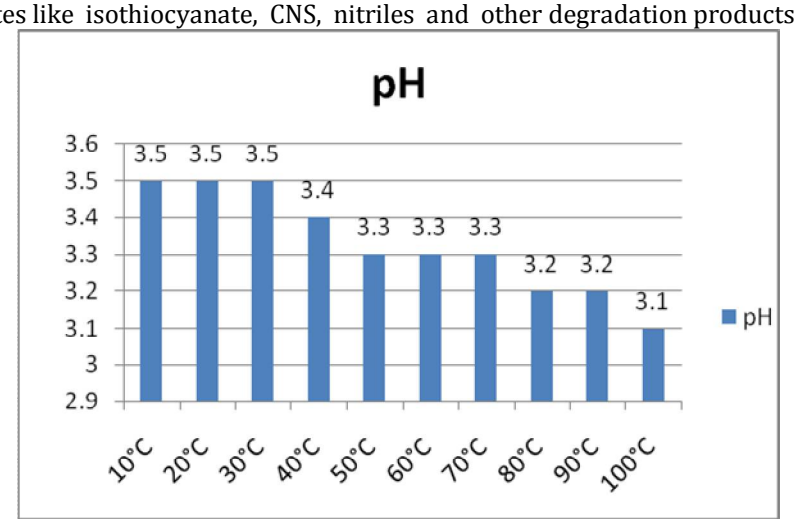

Figure 1.pH of KKKP at different temperature

The electrical conductivity of tested bases, formulate KKKP and DM were recorded as 3.1. ≥4.2≥3.2≥4.4≥2.4≥2.25≥2.2dS m^{-1}.Among the bases fish meal and cowdung have maximum Ec (≥4**dS m^{-1})**than others. Further the final endproduct KKKP EC is correlated with of chemical and found that EC depend on concentration of KKKP in the water range. undiluteld KKKP have the EC 8.25 Mhowhere as Rocket 44 EC have 0.0 Mho. Dilution of KKKP given 1.24, 2.15, 2.77, 3.48, 4.30 respectively for 1:9, 2:8, 3:7, 4:6 and 5:5 ratios (table 2). Similarly EC Rocket 44 also showed EC 0.29 ≥0.51 ≥0.77 ≥1.02 ≥1.19 Mho respectively at 1:100, 2:100,3:100, 4:100 and 5:100 dilution. The pH of chemical fertilizer was higher and EC was as low as KKKP (2.0). The electrical conductivity is indirectly proportional to pH. Sample with

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neutral pH have low EC than pH 8.Maximum viscosity of Base iii (Fish meal+ sprouts) alone have 0. 000798 followed by 0. 00581 by base IV and the end product KKKP viscosity is0.0006333255 which is equal to chemical pesticide. Base I and II also found to be slightly viscous. For various ions in solution, electrical conductivity can be used to determine soluble nutrients. Soil EC is an indicator of soluble nutrients that shows the metabolism of organic matter in soil. The electrical conductivity of soil increases dramatically with the application of poultry, cattle, and goat manures. The highest value of EC was found for cattle manure compost and the lowest value of EC was determined for the herbal plants residues compost reported by previous studies [13-14].

Table 1: Physiochemical parameters of KKKP and its bases

Sample	pH	EC (dS m^{-1})	viscosity
Base I cow products	8.6	4.2	0. 000324
Base II fruit waste+ sprouts	7.4	3.2	0. 000237
Base III Fish meal +jaggery	8.3	4.4	0. 000798
Base iv Plant /red algal extract	6.2	2.4	0. 00581
KKKP	3.5	2.25	0.0006333255
DM	6.0	2.2	0.000647088

TABLE 2. ELECTRICAL CONDUCTIVITY REPORTS

S.NO	SAMPLES (RAW SAMPLE)		ELECTRICAL CONDUCTIVITY (E)
1.	X-formula		8.25 Mho
2.	Rocket 44 EC		10.0 Mho
Ratio	**X-FORMULA**	**DIS.H_2O**	**CONDUCTANCE**
1:9	10 ML	90 ML	1.24 Mho
2:8	20 ML	80 ML	2.15 Mho
3:7	30 ML	70 ML	2.77 Mho
4:6	40 ML	60 ML	3.48 Mho
5:5	50 ML	50 ML	4.30 Mho
S.NO	ROCKET 44 EC	DIS.H2O	CONDUCTANCE
1:100	1ML	99 ML	0.29 Mho
2:100.	2ML	98 ML	0.51 Mho
3:100	3ML	97ML	0.77 Mho
4:100	4 ML	96 ML	1.02 Mho
5:100	5 ML	95 ML	1.19 Mho

Solubility and polarity nature of KKKP

Table 3 represents the formulated KKKP solubility and polarity nature. Solubility among Water, 5% $NaHCO_3$,5% NaOH, 5% HCL, Cold concentrated ofsulphuric acid and solvents were recorded. It was noted that the formulated organic compoundhighly soluble on 5% Hcl and Sulphuric acid but moderately soluble on other tested solvents. Soluble in acids indicates KKKP does behaving as an organic base. The report confirms the pesticide is insoluble under alkaline base due to presence of phenolic and organic acid properties. Solubility among acid denotes presence of various alkene and alcohols. Further the polarity of KKKP is extended to acetone in addition to water but not in methanol, hexane, toluene and benzene given non polar property. It failed to soluble in Hexane, Tolune, Benzene indicates high polar in nature.

Table 3: X-FORMULA SOLUBILITY TEST

S.NO	Acid and base solubility	Polar and nonpolar Solubility
1.	Water Partially soluble	Water Partially soluble
2.	5% NaoH-IS	Methanol-IS
3.	5% HCL-S	Acetone-S
4.		Hexane-IS
5.		Tolune-IS
6	-	Benzene-IS

IS- in soluble S- soluble

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Vapor pressure test

The quantified vapor pressure generated by KKKP is found that it produced 124K at 30º and reached maximum 141K at 90º C. The vapor pressure is low between 30-60ºC found to be ranges between 124-136K, elevating temperature may promote formation of some volatile compound which increase pressure. Vapour pressure of formulated liquid fertilizer (table 4) varies and dependent on temperature. At temperature below 60º C the pressure were less than 140K but reached maximum 141K at 90º C and drifted to 138 by raising temperature above 90º C. Many plants contain volatile chemicals that, when extracted, have fumigant properties and are toxic or repellant to many species of insects while being completely harmless to human. Essential oils' physical qualities, such as their high boiling point, molecular weight, and low vapour pressure, make them unsuitable for large-scale fumigation [15].

Table 4: Vapour pressure of the biopesticide under 5 ml syringe

S.no	Temperature	Volume taken	Vapour pressure
1.	30°	2ML	124K
2.	40°	2ML	128K
3.	50°	2ML	132K
4.	60°	2ML	136K
5	70°	2.1 ML	136K
6.	80°	2.1 ML	138K
7.	90°	2.1 ML	141K
8.	98°	2.2 ML	138K
9.	99°	2.2 ML	138K
10.	100°	2.2 ML	139K

Water Partition Coefficient of KKKP

In addition Lipophilicity is measured as the partition coefficient (Kow) of KKKP (Table 5) between non-aqueous (octanol, o) and aqueous (water, w) phases recorded as 1.14, 0.25, 0.27, 0.16 and 0.12, and is expressed as the logarithm of the ratios between these, or logP detected as 0.05, -0.6,-0.5,-0.79 and -0.9. Chemicals with very high logKow values (i.e, >4.5) are of greater concern because they may have the potential to bio-concentrate in living organisms. If the partition coefficient of product is more than one it is more lipophilic. It was detected that at 0.02 % its more lipophilic and increasing con affect the lipophilicity.

Table 5: Water Partition Coefficient of the Bio pesticide

S.NO	Volume of KKKP	AQUOUS PHASE OD VALUE	OCTANOL PHASE OD VALUE	Kow	Log p
1.	0.2	0.048	0.055	1.14	0.05
2.	0.4	0.014	0.055	0.25	-0.6
3.	0.6	0.015	0.055	0.27	-0.5
4.	0.8	0.009	0.055	0.16	-0.79
5.	1	0.007	0.055	0.12	-0.9

Carbon and Nitrogen ratio of KKKP

The data of table 6 reveals the C: N ration of final product KKKP is 17:1along with 72 % carbon and 4.2% nitrogen . Among the bases, base have high C/N ratio and estimated as 16:1. Base iv Plant and algal extract showed 5.2 C/N ratio with moderate of nitrogen content among bases. Base II showed C/N ratio 4.4:1 and base iii have 3.4:1respectively. Maximum nitrogen content was recorded in fishmeal base. Regarding the C/N ratio, it ranged from 3.4:1 to 17:1 for different component state. The lowest value of C/N ratio (3: 1) was found for sprouts +fishmeal compost and the highest value of C/N ratio (16:1) was noted on cow dung manure (fig 2). These results are in agreement with the results obtained by whose found that the C/N ratio ranged from 15:1 to 20:1 is ideal for ready-to use compost [16].According to Manivannan et al., (2009) adding organic material to soil immediately raises the amount of organic carbon in the soil, but with time, the amount of organic carbon in the soil declines up to a particular point [17].

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Table 6.Estimated Carbon and Nitrogen of KKKP

Sample	Organic Carbon %	Nitrogen %	C/N ratio
Base I cow dung	68	5	16:1
Base ii milk+fruit WASTE	22	5	4.4:1
Base iii Fish meal+ sprouts	29	8.4	3.4:1
Base iv Plant /algal extract	28.2	5.4	5.2:1
KKKP	72	4.2	17:1
Rocket 44 CC	6.4	1.2	5.3:1

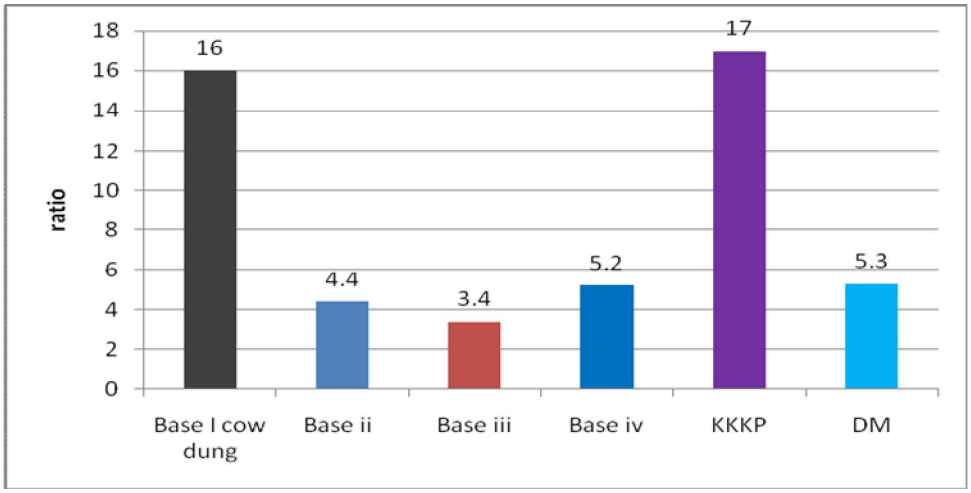

Figure 2.Ratio of carbon and nitrogen among KKKP base

Qualitative phytochemical analysis of plant and algal extract

Botanical pesticides are efficacious in managing different crop pests varied modes of action are attributed to the phytochemical composition in different plant. Qualitative phytochemical of KKKP base shows presence of various compounds out of 12 tested. Qualitative determination of phytochemicals of plant and algae is given in table 7 showed various plant secondary metabolites.

Table 7. Phytochemical Screening of Biopesticide

S.NO	TEST	Plant extract	Algal extract
1.	Coumarin	-	+
2.	Terpenoid	+	+
3.	Flavonoid	+	+
4.	Inulin	+	-
5.	Saponin	+	-
6.	Steroids	-	+
7.	Carbohydrate	+	+
8.	Tannin	+	+
9.	Alkaloid	+	+
10.	Lignin	-	+
11.	Protein	+	+
12.	Phenol	+	+
13.	Phloblatanins	+	+

Except coumarin, steroid and lignin all other phytochmicals like Terpenoid, Flavonoid, Inulin, Saponin, Carbohydrate, Tannin, Alkaloid, Lignin, Protein and Phenol were detected in plant extract base. In the present study, phytochemical screening of the seaweeds showed the presence of proteins, alkaloids, flavonoids, terpenoids, steroids, tannins, coumarins, terpenoids, quinine, phytosteroids and phlobatannins but it was noted that Saponin and inulin were absent. The presence of tannins shows that the plants can be used as purgative. They are also used in the treatment of cough, asthma and hay fever.

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The presence of terpenoids revealed that the plants can act mainly as anti-feedant and growth disruptor and possesses considerable toxicity toward insects. Phlobatannins and terpenoids were detected in the ethanolic extract phytochemicals may be responsible for their insecticidal properties [18]. Phenolic compounds are commonly found in plants, including seaweeds, and have been reported to show a wide range of biological activities[19].

Heavy metal analysis of KKKP

Determining micronutrients (Fe, Cu, Zn and Mn) and heavy metals (Cd, Pb, HgPb,Cr and Ni), samples were digested in HNO_3 and HCl in the ratio 1:3 and fed to atomic absorption spectrophotometer. Presence of toxic heavy metals Arsenic (As), Lead(Pb),Chromium and Nickel in the final KKKP mass is ranged at permissible level recorded as 0.322≥0.12l≥0.1≥0.08 mg/L. Arsenic (As) and Cadmium(Cd) were not detected(table 8).Presence of minute content of these toxic heavy metals noted in the study might be the contamination from the soils. Concentrations of trace metals among the KKKP samples had shown the descending order of Zn≥ Fe>Mn>>Cu and estimated as 1. 522≥1.132≥0.615≥0.350 mg/L (table 9). Lwin et al(2012) reported presence of heavy metal content of liquid organic fertilizer have inhibitory effect on germination and growth of young seedlings. Fertilizers, including organic and inorganic elements, are responsible for producing heavy metals in the soil [20]. Presence of Cd, Pb, Cu, Zn among livestock manure around the world. A high concentration of Pb can cause different physiological and biochemical deficiencies.

Table 8. Heavy metal analysis of KKKP

Parameters	Nm	Mg/L
Arsenic (As)	193.696	0.322
Cadmium(Cd)	228.802	Not Detected
Mercury (Hg)	253.652	Not Detected
Lead(Pb)	220.353	0.127
Chromium	357.869	0.1
Nickel	232.003	0.008

Table 9. Mineral analysis of KKKP

Copper (Cu)	327.393	0.350
Manganese(Mn)	257.610	0.615
Iron(Fe)	238.208	1.132
Zinc(Zn)	213.851	1. 522

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