OPTIMIZATION THE EFFICIENCY OF ORGANIC AMENDMENTS FOR REMEDIATION OF INSECTICIDE-CONTAMINATED SOIL

May Thet Tun[1], Phyu Phyu Myint[2], Saw Hla Myint[3]

Abstract

The main aim of this research is to examine the optimization of the organic amendments for remediation of insecticide contaminated soil. The waste materials for crude protein contents, fish scale (*Cirrhinus cirrhosus*), chickpea shell *(Cicer arietinum* L.*)* and peanut shell (*Arachis hypogaeal* L.) were collected from Pyay Township, Bago Region. The crude proteins were extracted from the selected sources by trichloroacetic acid (TCA) method, and evaluated the yield % of nitrogen. The nitrogen contents of fish scale, chick pea shell and peanut shell were found to be 1.527 %, 0.901%, and 0.990%, respectively. Furthermore, the protein extract from selected sources were confirmed by chemical tests. According to the result, the fish scale waste was found to possess the highest content of protein. The fish scale could be potentially applied as a nitrogen source in the determination of soil enzyme activity and degradation of insecticide in soil. Before treatment, two types of amendments were prepared by fermentation for 10 days on mixing the samples viz., FSJ (fish scale-FS: Jaggery-J - 10:10 w/v) and FST (FS: Treacle-T - 10:10 w/v). A laboratory bench study was conducted to assess the removal efficiency of FSJ and FST on 1 ppm insecticide contaminated soil. Three different nitrogen additives from two natural sources; FSJ, FST and one chemical, NH_4-N source with a concentration 4%each treatment were applied on insecticide contaminated soil along with control (no treatment). The residual insecticide (as its metabolite 3-phenoxybenzoic acid 3-PBA) in soil samples extracted from the experimental plot was examined using UV-vis spectrophotometer. After five weeks treatment, the removal efficiency of FSJ and FST treatments was found significantly increased. The profile of soil urease activity in the treated and contaminated soil was also determined by Phenol-Hypochlorite method. The overall results indicated that appropriate waste- amendment application can promote the removal of PBA as well as maintain the activity of urease enzyme in soil.

Keywords: protein extraction, TCA method, waste amendment effect, contaminated soil, urease activity

Introduction

Nowadays, with global population exceeding seven billion, agriculture inexorably continues to play a very important role in the survival of mankind. Since many years, farmers have been used insecticides to kill unwanted insects and to control pests that infest crop. Nearly all insecticides have the potential to significantly alter ecosystems: many are toxic to humans and/or animals; some become concentrated as they spread along the food chain. The presence of these chemicals in both aquatic and terrestrial ecosystems has become an important issue globally. Agriculture and Toxicology provides information on the use of insecticides in pest management in order to enhance crop protection and their effects on nontarget organisms. Insecticides are agents of chemical or biological origin that control insects (Amweg *et al*., 2005). The widespread use of insecticides over the past 30 years has resulted in problems caused by their interaction with natural biological systems. The persistence of insecticides and their degradation products depends on how deeply they are mixed into the soil; even the most persistent compounds disappear relatively quickly when on the soil surface, yet when incorporated into the soil they are very persistent (Dileep, 2002).

Nitrogen (N) fertilizer application is an important measure to improve the soil fertility and crop yield, also it is one of the most management measure in agriculture. Soil microorganisms are important components of soil ecosystem, leading the nutrient cycle and energy flow meanwhile, it

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plays an important role on the ecosystem stability and sustainability (Jingjing *et al*., 2015). Urease is the enzyme that degrades urea and is widely considered to be a good proxy of nitrogen (N) mineralisation. Urease is widely distributed in soils and was one of the first soil enzymes to be experimentally evaluated (Cordero *et al.,* 2019).

The aim of the present research work is to study the effect of nitrogen on remediation of pyrethroid insecticides in contaminated soil and determine the soil urease activities in the treated and contaminated soil.

Materials and Methods

The fish scale, chickpea shell and peanut shell were collected from Pyay market, Pyay Township, Bago Region and identified at Zoology and Botany Department of Pyay University. They were dried, ground well with a pestle and mortar into powder and stored in air-tight container. Figure 1 shows the collected samples used in this study. Treacle from Mya Ywar Sugar Factory, and jaggery residue from Tamikethar village were also collected.

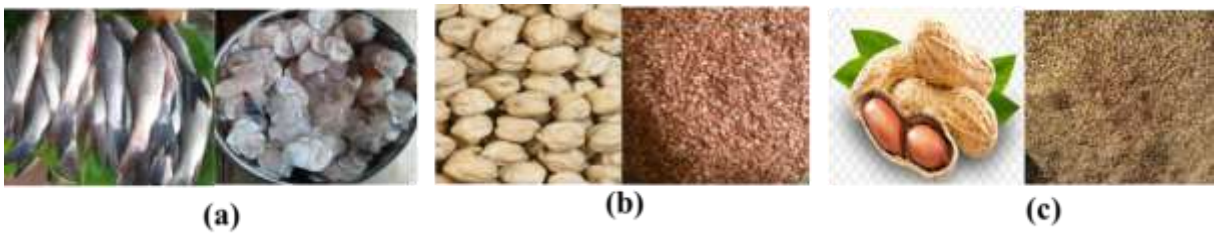

Figure 1 Photograph of samples: (a) fish and fish scale (b) chickpea and chickpea shell (c) peanut and peanut shell

Extraction of Protein from Waste Samples (TCA Method)

Dried powdered sample (1 g) was accurately weighed and extracted with an aliquot of ethanol: petroleum ether (2:1v/v) mixture with constant stirring for 15 min at 550 rpm. The sediment was washed again with pet-ether (200 mL) and stirred 15 min at 550 rpm. The residue was dried at room temperature to remove all traces of pet-ether and dissolved in 5-10 % trichloroacetic acid (TCA). The mixture was kept at 4ºC for about 4 h and filtered. Then 1M NaOH (100 mL) was added into the residue and stirrred for 1h at 550 rpm and filtered. After filtration, 3M HCl was added to the mixture and pH was adjusted to 4.2 and filtered. Next, 1M NaOH was added to get pH 9.0 and stirred for 1h at 550 rpm and filtered. After that, 3M HCl was added to adjust pH 4.2 and filtered. The residue was calculated to obtain percentage of pure protein.

Characterization of crude protein

The extracted crude protein was characterized by Qualitative tests for protein such as Xanthoproteic Test, Biuret Test and Millon's Test.

In Xanthoproteic Test, 2 mL of crude protein solution was taken in a test tube and 0.5 mL of concentrated HNO_3 was added and boiled. The mixture was cooled under tap water and concentrated ammonium hydroxide was added to make the solution alkaline. In Biuret Test, 3 mL of crude protein solution was taken in a test tube and added an equal amount of 10 % sodium sulphate solution. In Millon's Test, about 5 mL of protein solution was taken in a test tube and a few drops of Millon's reagent were added. After mixing thoroughly, the mixture was heated to the boiling point.

Collection of Soil Sample

The soil was collected from the surface layer (0-20 cm) of an agricultural field located in Tamikethar village (at 21°50′ latitude north and 96°40′ longitude east), Myingyan Township, Mandalay Region (Figure 2).

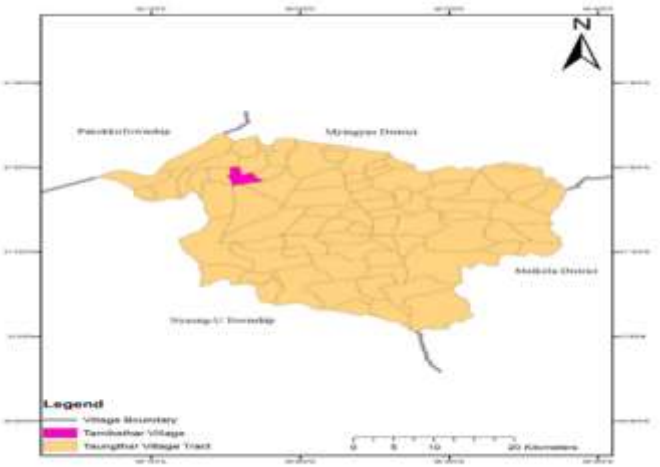

Figure 2 Location of Thamikethar Village

Determination of Physicochemical Properties of Soil Sample

The moisture content of the soil samples was determined according to the reported method. pH content of the soil sample was determined according to the standard method by using pH meter. EC content of the soil sample was determined by using electrical conductivity meter. Organic matter content of the soil sample was determined by using Walkley and Black method based upon the oxidizable organic matter content. Total N content was determined by using Kjeldahl's method. Cation exchange capacity content of the soil samples were determined according to the method of Kappen. Total P of the soil samples was determined by using Olsen method for neutral and alkaline soil (measured by spectrophotometer). Potassium content of the soil samples was determined by using ammonium acetate extraction method (measured by Flame photometer).

Experimental Design

Organic Amendment Samples Preparation

To make the organic amendment, 20 g of fish scale powder was put in a beaker, treacle or jaggery residue of an equal amount (1:1 weight ratio) was added, then mixed thoroughly using a glass rod. The beaker was covered with aluminium foil. The fish scale will have fermented in 14 days' period. The solution was extracted and used as organic amendments; FSJ and FST.

Determination of Insecticide-contaminated Soil Remediation

Fresh soil samples, each equivalent to 10 g of dry soil, were placed in 150 mL beakers and their water contents were adjusted to 60% of water holding capacity. Each treatment in triplicate was spiked with 1 mL cypermethrin (1000 µg/ml). Nitrogen source was added in the form of Nitrate-N by using nitrate fertilizer. FSJ and FST dissolved in water were also added separately into different beakers. All containers were covered with aluminium foil to ensure gas exchange and then incubated at 25 °C for 0 week, 1 week, 2 weeks etc. and up to the 5 week.

Determination of Insecticide Removal

The residue of insecticide was extracted from soil samples (10 g) using the reagent of methanol: dichloromethane (3:1 v/v). Each sample was shaken at 200 rpm for 15 min and then centrifuged at 5000 rpm for 15 min. Solution aliquots were then analyzed using an ultraviolet-visible (UV-vis) detector.

Determination of Urease Activity

The urease activity was determined by urea reduction method. Firstly, 10 g of fresh soil was placed in a 100 mL volumetric flask and treated with 1mL of toluene, 10 mL of buffer (pH-7) and 5 mL of 10 % urea solution (freshly prepared). After a thorough mixing the flask was incubated for 3 h at 37 ºC in dark. For the control, 5 mL of 10 % urea solution was replaced by 5 mL of sterile distilled water. After incubation the volume of the flask was made up to 100 mL with distilled water and shaken thoroughly and transferred the filtrate through Whatman No. 5 filter paper. The ammonia released as a result of urease activity was measured by indophenol blue method. In brief 0.5 mL filtrate was taken into a 25 mL volumetric flask and 5 mL of distilled water was added. Then 2 mL of phenolate solution (mixture of 20 mL of stock A (62.5 g phenol crystals dissolved in a minimum volume of methanol and made up the volume up to 100 mL with ethanol after adding 18.5 mL acetone and 20 mL of stock B (27 g NaOH dissolved in 100 mL distilled water and kept in freezer)) were added. Thereafter, 1.5 mL of sodium hypochlorite solution was added. The final volume of the flask was made up to 25 mL with distilled water and the blue colour was read out with the spectrophotometer at 630 nm.

Results and Discussion

Extraction of Protein from Waste Samples

The crude protein contents of fish scale, chickpea shell and peanut shell were found to be 1.527 %, 0.901 %, and 0.990 % respectively. According to this result, the fish scale waste was found to possess the highest content of protein.

Table 1 Protein Contents of Selected Waste Extracts

Sample	Protein content(%)
Fish scale	1.527
Chickpea shell	0.901
Peanut shell	0.990

Confirmation of the extracted protein from waste samples

In Xanthoproteic test, the crude protein samples extracted from the fish scale, chickpea shell and peanut shell were observed as yellow colour solutions**.** This observation inferred that amino acids like tyrosine, tryptophan, and phenylalanine were present in these waste samples. In Biuret Test, all extracted crude proteins were observed as violet colour solutions. So, these waste samples were indicated the presence of albumin. The intensity of the colour increased as the number of linkages involved in the proteins increased. The fish scale in Millon's Test did not give brick red colour whereas chickpea shell and peanut shell gave brick red precipitate. It was found that all samples gave positive results for Biuret test. These samples gave purple colour indicating the presence of proteins. However, tyrosine are present in chickpea shell and peanut shell according to Millon's test.

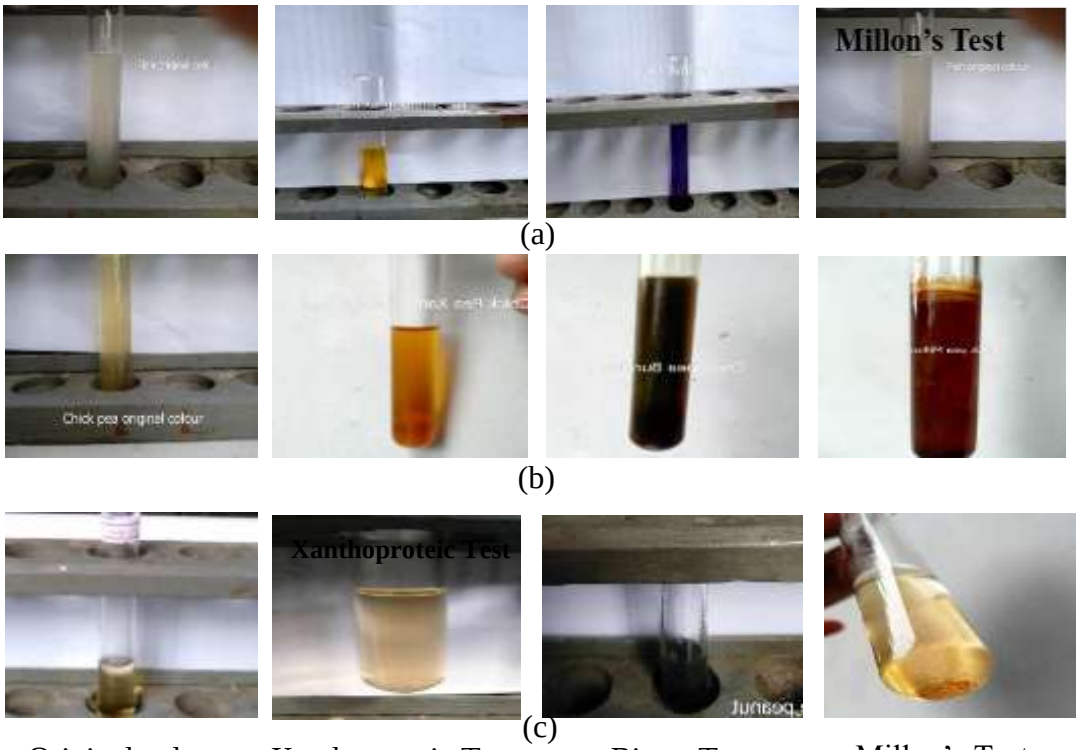

Figure 3 Photographs of confirmation tests for crude protein extracted from (a) fish scale sample (b) chickpea shell and (c) peanut shell samples

Physicochemical Properties of Soil Sample

The characteristics and physicochemical properties of soil sample are shown in Table 2. Soil pH value was found to be 8.22. The representative soil that used in this study was the type of moderate alkalinity. The data pointed out that the moisture content was 4.75 %. The moisture content of soil sample was sufficient for cultivation of plant. Moreover, 0.87 % of humus and 0.50% of organic carbon contents were found in the representative soil and it was found to have very low in organic matter content. The experimental data showed high in Ca (26.61 meq/ 100 g), medium in Mg (2.10 meq/100 g), low in K (0.62 meq/100 g) and Na (2.99 meq/100 g). The concentration of calcium helps in membrane stability maintenance of chromosome structure and enzyme inhibitor. Magnesium helps the movement of sugar within plant. Sufficient amount of potassium increases the size of grains or seeds and improves the quality of fruits and vegetables. The deficient K causes the shriveled seeds or fruits. Sodium is not essential element for plants but can be used in small quantities, similar to micronutrients, to aid in metabolism and synthesis of chlorophyll. The contents of total nitrogen, phosphorus and potassium were 0.09 %, 7.56 ppm and 28.99 mg/100g, respectively. An abundance of nitrogen promotes rapid growth with a greater development of green leaves and stems. The presence of sufficient available phosphorus is required for seed formation and crops maturity.

Table 2 Characteristics of the Soil Sample

Parameter		Content
Moisture (%)		4.75
pH Soil: Water 1:2:5		8.22
EC (mS/cm)		0.10
Organic Carbon (%)		0.50
Humus (%)		0.87
Total N (%)		0.09
CEC (meq/100 g)		32.32
Exchangeable cations	Ca^{2+}	26.61
	Mg^{2+}	2.10
	K^{+}	0.62
	Na^{+}	2.99
	H^{+}	Not detected
	Al^{3+}	Not detected
Available nutrients	P (ppm) (Olsen)	7.56
	K_2O (mg/100g)	28.99

Determination the organic amendments efficiency on soil remediation

The insecticide residue in soil was observed to be decreased gradually in all treatments after 5 - week period. It was found that the urea, FSJ and FST treatments significantly increased the degradation of cypermethrin in soil as compared with the control (no treatment) after 2-week period. After 5 weeks, the removal efficiency of FSJ and FST treatments were found significantly increased. These findings indicated that adequate addition of N could increase the degradation of cypermethrin but oversupplying N suppress it. However, the degradation levels were similar after a long enough incubation period.

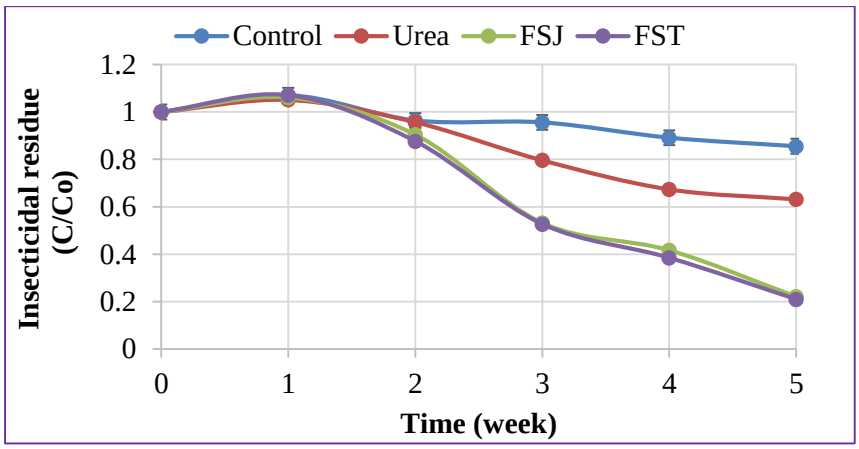

Figure 4 Insecticide residues in soil by different dosage of various amendments treatments

Enhancement of Urease Enzyme Activity

According to comparative study of all treatments, it was found that the urease activity was significantly increased by FSJ and FST treatments more than the control (no treatment) after 2-week and 3-week periods. The maximum urease activity levels were observed in the urea, FSJ

and FST treatments after 4 and 5 weeks. These results suggested that adequate additions of N stimulated soil microbial activity, which in turn enhanced degradation of cypermethrin.

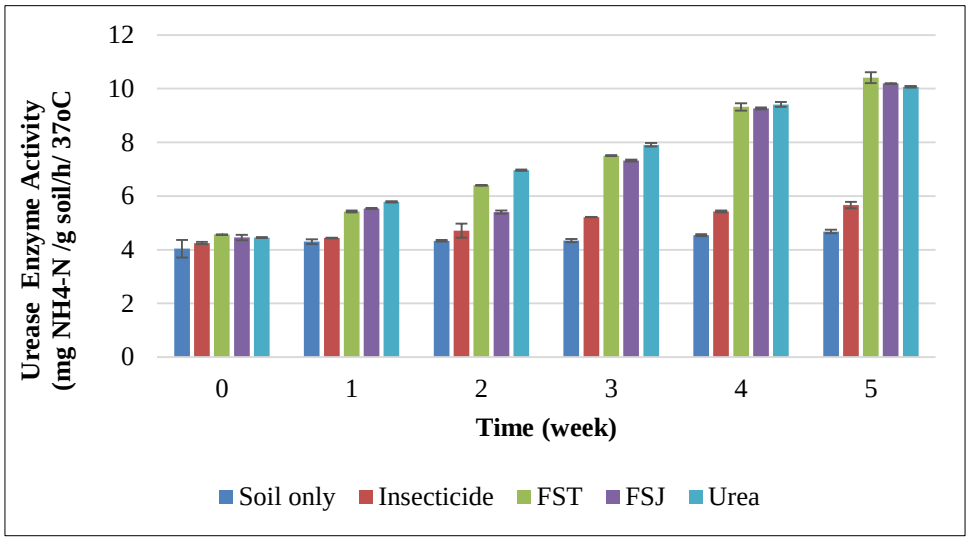

Figure 5 Urease enzyme activity of insecticide contaminated soil

Conclusion

The crude proteins were extracted from different waste materials, fish scale, chickpea shell and peanut shell by trichloroacetic acid (TCA) method, and the protein contents were found to be 1.527 %, 0.901% and 0.990%, respectively. Furthermore, the confirmation tests of protein in selected sources were done. According to this result, the fish scale waste was found to possess the highest content of protein. The fish scale could be potentially applied as nitrogen source in the determination of soil enzyme activities and remediation of insecticide in soil. The results of this study showed that addition of N at an adequate rate could enhance remediation of cypermethrin and its most persistent metabolite, PBA. Thus, in agricultural practice, adequate application of N fertilizer was an efficient method to reduce the accumulation of cypermethrin and PBA in soil and significantly decreased environmental risks, oversupplying N inhibited the degradation of cypermethrin and PBA in soil.

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