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A Study on the Removal of Pesticide Residues on Potatoes Using *Moringa oleifera* Seed as a Food Safety Measure

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***Abstract*— *Moringa oleifera (MO) is a plant whose seeds possess strong coagulation properties capable of removing turbidity and heavy metals from water. In this study, the effectiveness of MO seeds as a potential pesticide removal apparatus for agricultural produce was investigated. The parameters of interest for each pesticide washing solution were pesticide-concentration reduction, cost, and taste alteration to the potatoes. Separate doses of 0.25 mL profenofos 500 EC were individually applied to distinct 250 g pesticide-free potato piles. A submersion of each tainted potato pile was carried out in one of the chosen pesticide baths (mineral water, 2% salt solution, 10% salt solution, vegetable bath wash, and 10 g MO seed) for 10 min, followed by a 10 second mineral water bath rinse. Remaining profenofos residue concentrations on potatoes were quantified using the quick, easy, cheap, rugged, and simple QuEChERs method coincided with High Performance Liquid Chromatography (HPLC). The experiments showed that the MO seed was the most effective at removing profenofos at 52.9% followed by the vegetable bath at 47.3%. Neither method caused an alteration in food taste, but the MO seed method is significantly cheaper at 2,000/L IDR ($0.14 USD) compared to the vegetable bath at IDR 10,668/L ($0.77 USD) at the time of the experiment, respectively.*

Keywords*— *Moringa oleifera, pesticides, profenofos, coagulation, agriculture.

I. INTRODUCTION

Over application of pesticides in agriculture has been an ongoing issue in Indonesia. Farmers who tend to administer copious amounts of it onto their crops do not view pesticides as a poison, but as nurturing medicine; and that superfluous application will only harness advantageous benefits (Shepard, 2008). These health concerns propelled the government to pursue solutions on behalf of its people. Indonesia united with the Food and Agricultural Organization (FAO)'s Codex Alimentarius Commission in 1971; which includes recognizing FAO's maximum residue level (MRL) guidelines. In addition, the country also established an integrated pest management (IPM) program in 1993 (Feder *et al*., 2003). The IPM program, with funding provided by the World Bank, offered educational pesticide-reduction methods for farmers to implement, which not only lowered the pesticide usage, but inversely increased crop yields as well. However, the program ended in 1999 due to a loan termination (Resosudaro, 2001). This has left a share of farmers unaware of the practice and in turn, past ideology on pesticide usage still remains in certain regions.

Profenofos (O-4-bromo-2chlorophenyl O-ethyl S-propyl phosphorothioate) is an organophosphate pesticide commonly used in Indonesia to rid pests from agricultural produce due to being legal, cheap, and easily accessible. FAO warns that high dosages the pesticide can inhibit cholinesterase activities in red blood cells, plasma, and the brain. As high concentrations of profenofos continue to enter water bodies caused by agricultural runoff, aquatic life becomes more vulnerable to the previously mentioned health risks; due to oxygen being depleted while the concentration of pesticide increases inversely. (A. Maharajan). Indeed, non-toxic pesticide washing solutions have been created by (Abou-Arab, 1999; Rao *et al*., 2014; Kelageri *et al*., 2015) which can successfully reduce large concentrations of profenofos from agricultural produce. However, the preparation costs may deter Indonesians who earn very low incomes from accepting the practice. Furthermore, all the articles failed to include any factor of taste into their studies. Regardless of how successful a reagent is, if it causes a food crop to develop a foul taste, it will not be continuously endorsed.

Moringa oleifera (MO) or kelor in Bahasa Indonesia, is a plant entwined into the cultural fabric of Indonesia. It is used as a food and herbal crop, carries mystical powers according to local legend, and has presence in a famous idiomatic expression. Moreover, MO seeds have been

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routinely used as a simple, yet effective water treatment apparatus; especially since no additional reagents are required. Validation of this efficacy is conducted from studies demonstrating its coagulation potential on removing turbidity and heavy metals from water (Amagloh *et al*., 2009; Katayon et *al*., 2005; Nand *et al*., 2012; Nkuunziza *et al*., 2009; Obuseng *et al*., 2012). Using both computer software to evaluate the mechanisms responsible for the catatonic protein plus azo dyes and dichlorodiphenyltrichloroethane for *in situ* investigation, researchers have concluded that the MO seed would be capable to bond itself to pesticide molecules (Pavankumar *et al*., 2014).

There is a deficiency of scientific literature investigating the potential of MO seed as a successful reagent to remove pesticides. As a result, this study will address this topic by using profenofos as a means of attempt. Findings were compared against other pesticide washing methods, which successfully remove profenofos, to determine removal percentage significance. Additional branches of analysis including an added taste test with a focus group and cost production factors were also considered.

II. MATERIALS AND METHODS

2.1. Reagents and chemicals

For sample preparation, KSO-8909 roQ QuEChERS Extraction Kit EN Method containing Magnesium Sulfate ($MgSO_4$), Sodium Chloride (NaCl), Sodium Citrate Tribasic Dihydrate (SCTD), Sodium Citrate Dibasic Sesquihydrate (SCDS), and KSO-9507 roQ QuEChERS d-SPE Kit containing Magnesium Sulfate ($MgSO_4$) and 150 mg Primary/Secondary Amine (PSA) were purchased from Phenomenex (Torrance, CA, USA). HPLC grade water, formic acid, acetonitrile (ACN) and sodium hydroxide (NaOH) pellets were obtained from P.T. Smart Lab Indonesia (South Tangerang, Banten, Indonesia). Profenofos 500 emulsible concentrate (EC) and potable mineral water were procured from the local market.

2.2. Standard compound preparation

Profenofos 500 EC was used to prepare pesticide standards of 10 µg/mL and 40 µg/mL in ACN containing 0.1% formic acid. Determination of profenofos was accomplished by retention time (Rt) through HPLC and distinguished amongst the other chromatographic peaks by utilizing the established profenofos flow rate (mL/min) in (Sanagi, 2008). With guidance as such, the retention time of profenofos was detected at 29 min. An internal standard was considered unnecessary and therefore omitted from the study.

2.3. *Moringa oleifera* seed preparation

MO seeds were procured from the local market, already separated from the fruit pods and dried. The seeds were tested for effectiveness by reflecting on the methodology in (Pavankumar *et al*., 2014). 3 drops of azo dye Ponceau 4R C.I. 16255 were added to 3 separate 1.5 L plastic bottles containing 1 L tap water to simulate a turbid water environment. Dosages ranged from 1 to 2 seeds, with preparation requiring their seed coats and wings from the cotyledons (seed kernels) to be removed and then crushed into a powder using a mortar and pestle. The powder from 1 seed was added to the first bottle, the powder from 2 seeds were added to a second bottle and the third bottle remained as the control. After a 24hr period and again after 1 week, coagulation was established by visually analyzing the reduction in color and translucence of the solutions containing the MO seeds against that of the control. Figure 1 below displays the collected visuals taken during the *in situ* test.

A. Initiation of Test

B. Results after 24 hours

C. Results after 1 week

Fig 1: Coagulation visuals of the Moringa oleifera seed effectiveness test. Each image contains each type of bottle in the following order: far left containing 0 seeds, the middle with 1 seed and the far right with 2 seeds.

2.4. Decontamination Methods

Quick absorption of profenofos into the skin of a food crop was essential for this study. The adoption of potatoes for this experiment was ideal due to their porous skin along with profenofos commonly used to treat pests from the root vegetable. Pesticide-free potatoes were purchased in 1 kg/sac from local grocery stores whose stocks are procured from the same distributor. Each potato was weighed and

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then sorted to form numerous piles weighing 250 g with a lenience of ± 5 g. This process allowed the potatoes to be successfully organized while remaining uncut and intact. Afterwards, each potato pile was placed in a separate plastic bag and set in the laboratory refrigerator at 8°C until needed.

To apply the pesticide to the potatoes, 0.25 mL of the stock profenofos was mixed with a couple droplets of deionized water to formulate a paste, and then coated all over the potatoes' skin using a basic paintbrush in the fume hood at a room temperature of 21°C. The potatoes were then immediately placed into the decontamination baths that were replicated procedures of the most successful washing solutions found in (Abou-Arab, 1999; Rao *et al*., 2014; Kelageri *et al*., 2015); with minor alterations, plus the MO seed bath incorporated. Glacial acetic acid was substituted with 25.0% (aq) vinegar which was diluted to achieve the 4.0% value. Because the tap water in Indonesia is non-potable, purified mineral water was used. To reduce the cost associated with this study, the volume of mineral water used for each bath was reduced from 4 L to 500 mL. Refer to Table 1 for a detailed list of the pesticide bath procedures.

Table 1: Protocol of pesticide decontamination bath types.

Bath 1	250 ± 5 g of potatoes were dipped in a plastic tub of 500 mL mineral water for 10 min, and then rinsed in a separate plastic tub of 500 mL mineral water for 10 sec.
Bath 2	Vegetable bath wash was prepared by mixing in a plastic tub: 1 g baking soda, the juice of 1 lemon and 80 mL of 25% vinegar in 500 mL mineral water, followed by dipping 250 ± 5 g of potatoes in the bath for 10 min, and then rinsed in a separate plastic tub of 500 mL mineral water for 10 sec.
Bath 3	2% salt solution was prepared by mixing in a plastic tub: 20 g table salt in 500 mL of mineral water, followed by dipping 250 ± 5 g of potatoes in the bath for 10 min, and then rinsed in a separate plastic tub of 500 mL mineral water for 10 sec.
Bath 4	10% salt solution was prepared by mixing in a plastic tub: 100 g table salt in 500 mL of mineral water, followed by dipping 250 ± 5 g of potatoes in the bath for 10 min, and then rinsed in a separate plastic tub of 500 mL mineral water for 10 sec.
Bath 5	*Moringa oleifera* seed bath was prepared by blending 10 g (about 48 seeds) of deshelled MO seeds in 500 mL of mineral water until a homogenous mixture was present and then poured into a plastic tub, followed by 250 ± 5 g of potatoes being dipped in the bath for 10 min, then rinsed in a separate plastic tub of 500 mL mineral water for 10 sec.

2.5. Extraction and Clean-up

Samples were extracted and cleaned up using European Committee for Standardization method EN 15662, Quick Easy Cheap Effective Rugged and Safe (QuEChERS) methodology. 10 ± 0.1 g of the potato puree was added into a 50 mL polyethylene centrifuge tube using a spatula. After adding 10 mL of ACN to each tube using a solvent dispenser, they were sealed well and shook vigorously by hand for 1 minute to ensure that the samples were thoroughly homogenized. The contents from one of the roQ extraction packets found inside the roQ QuEChERS extraction kit was dispersed into each centrifuge tube. Each extraction packet contains the following: 4 g $MgSO_4$, 1 g NaCl, 1 g SCTD, and 0.5 g SCDS. 400 µL of a saturated NaOH aqueous solution was also added to increase the pH of the sample to about 5.5. The tubes were sealed well again and the shaking procedure was repeated for 1 minute. The extraction process was completed by centrifuging the potato samples for 5 minutes at 4,000 rpm at 6°C; to allow proper separation between the liquid and solid particles. 6 mL of the aliquot from the supernatant was transferred into the 15 mL tube containing d-SPE sorbents 900 mg $MgSO_4$, and 150 mg PSA for cleaning the sample. The shaking process and centrifugation process was carried out again as previously mentioned. A portion of supernatant was extracted, filtered with a nylon syringe filter, and then transferred into a vial for HPLC analysis.

2.6. Residue Determination

The HPLC analysis was performed using model AGILENT 1100 Series HPLC system using manual injection equipped with quaternary pumps, diode array detector, and a Zorbax Eclipse XDB-C18 (4.6 x 150, 3.5 µm particle size, Agilent Technologies, Inc.). Mobile phases were A: 5mM formic acid in water, and B: 5mM formic acid in ACN at flow rate of 0.3 mL/min. The column temperature was 25°C, injection volume 10 µL, and the wavelength was set at 220 nm. ChemStation software was used to acquire the data.

The gradient program adhered to the methodology of *Leung et al*., (2012) with modifications: 0-15 min, phase A was 75% (v/v) and phase B was 25% (v/v); 15-30 min, phase A was reduced from 75% (v/v) to 10% (v/v) and phase B was increased from 25% (v/v) to 90% (v/v); 30 min, phase A was returned to 75% (v/v) and phase B was returned to 25% (v/v). Finally, from 30-40 min both phase A and phase B were maintained to complete the analysis.

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2.7. Focus Group Taste Test

Pesticide-free baby potatoes were purchased from the local market and used for the taste test. Being able to take advantage of their small size, preparation of the vegetables into bite-sized servings was not required. Furthermore, with the potatoes remaining intact each participant would involuntary taste the skin of the potato firstly. Indeed, since the skin is to be most affected under pesticide bath conditions, keeping the potatoes encased in its own skin allows the taste buds on the tongue a greater chance of detecting any potential taste abnormalities. The bath-protocol produce for baby potatoes complied with the procedures as mentioned earlier sans pesticide application and conducted in new, separate tubs. Next, they were placed on culinary metal trays, labeled, and baked in a food-grade laboratory oven at 190˚C for 20 minutes. Once cooked, the trays of baby potatoes were removed and lined side-by-side on the table. Once they cooled to room temperature, twelve test participants were brought in and asked to sample a potato from each tray. The tray containing the baby potatoes that had been subjected to the mineral water bath was transparently labeled while the other trays were assigned an identification number (T#) for concealment purposes. Their task was to point out any taste irregularities in comparison to that of the mineral bath potato tray; as the control group. The participants were allowed to sample the control group and drink water as necessary to cleanse their palate so that their sampling capabilities remained refined. Swallowing the potatoes was prohibited, for a feeling of fullness or nausea from eating could impair the participant's judgement. As a result, after sampling, the potatoes were discarded into a waste receptacle. Detailed results are displayed in Table 2.

Table 2. Results from the focus group taste test.

Bath ID.	Bath type	Taste sameness	Taste difference	Description of difference
Control	Mineral water wash	N/A	N/A	N/A
T1	2% salt solution	12	0	N/A
T2	10% salt solution	10	2	Slightly salty
T3	Vegetable wash	12	0	N/A
T4	*Moringa oleifera* seed solution	12	0	N/A

III. RESULTS AND DISCUSSION

The potato sample that had undergone just pesticide application, achieved a milli absorbance unit (mAu) peak area value of $4.41523e^4$ at a retention time (Rt) of 28.337. This mAu was established as the baseline value to behold and resolve the reduction values akin to the utilized pesticide baths. The potato sample associated from the mineral water bath accumulated a peak area of $2.51274e^4$ at Rt = 28.864 min., the 2% salt solution 2.91567e4 at Rt = 28.743 min, the 10% salt solution $2.68123e^4$ at Rt = 28.738 min, the vegetable bath $1.73966e^4$ at Rt = 28.784 min, and the MO seed bath $1.65515e^4$ at Rt = 28.795 min; which attained reduction percentage efficiencies of: 43.09%, 33.96%, 39.27%, 60.59%, and 62.51% respectively. Chromatograms of profenofos separation by HPLC sample analyses are depicted in Figures 2-7. A side-by-side visual depiction of the various pesticide remediation bath results are presented in Figure 8.

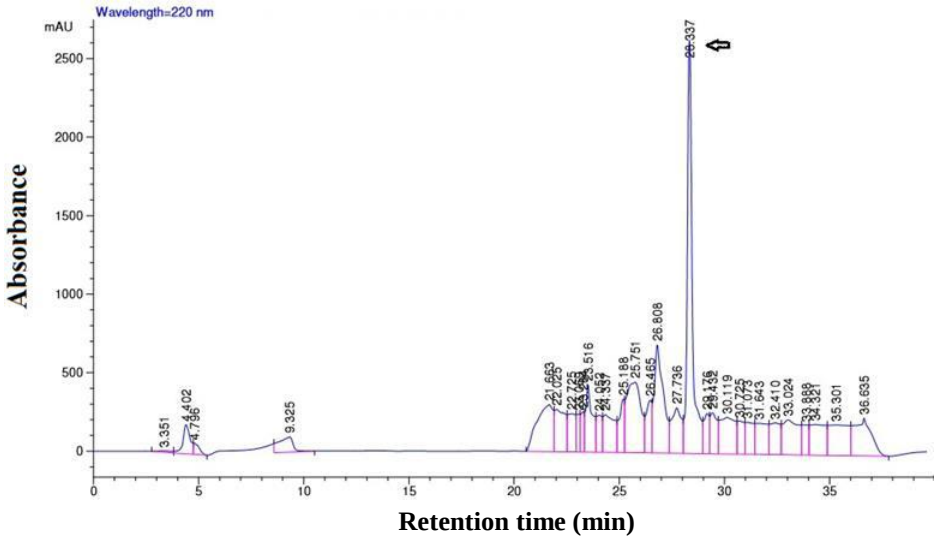

Fig 2: HPLC chromatogram of sample unexposed to any washing method. Profenofos (28.337 min) is identified.

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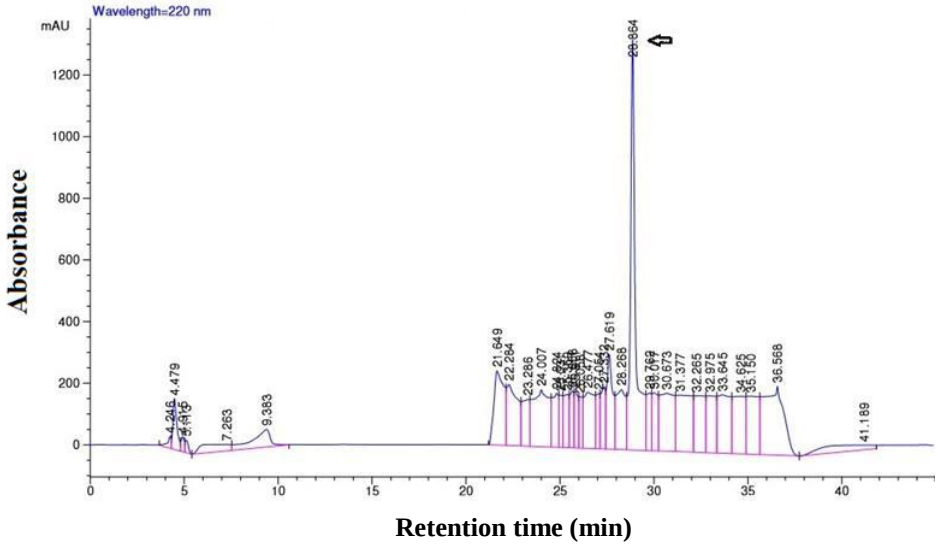

Fig 3: HPLC chromatogram of sample exposed to mineral water bath. Profenofos (28.864 min) is identified.

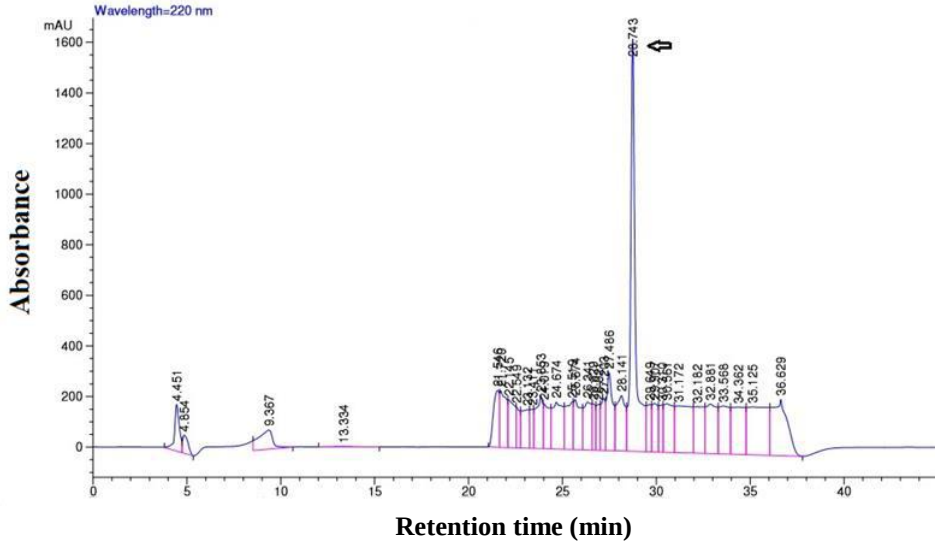

Fig 4: HPLC chromatogram of sample exposed to 2% salt solution. Profenofos (28.743 min) is identified.

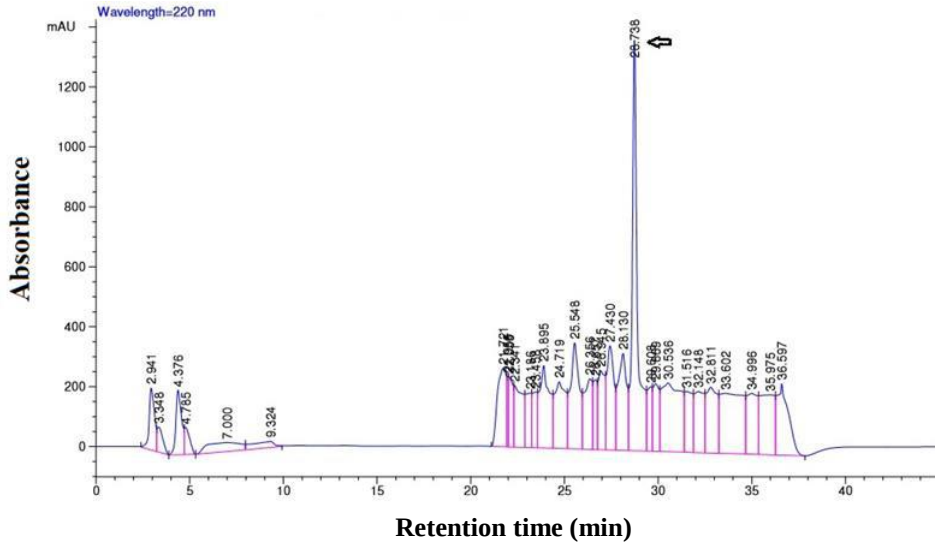

Fig 5: HPLC chromatogram of sample exposed to 10% salt solution. Profenofos (28.738 min) is identified.

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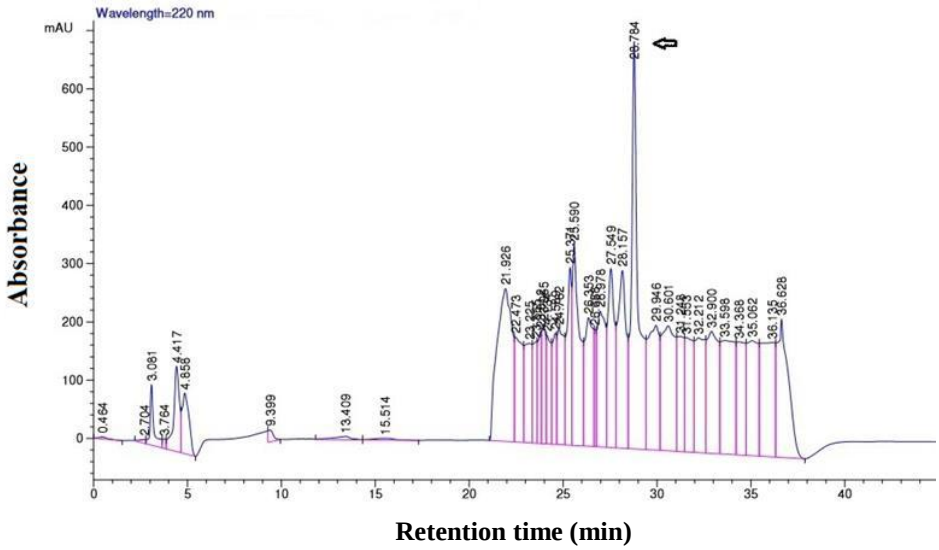

Fig 6: HPLC chromatogram of sample exposed to vegetable wash. Profenofos (28.784 min) is identified.

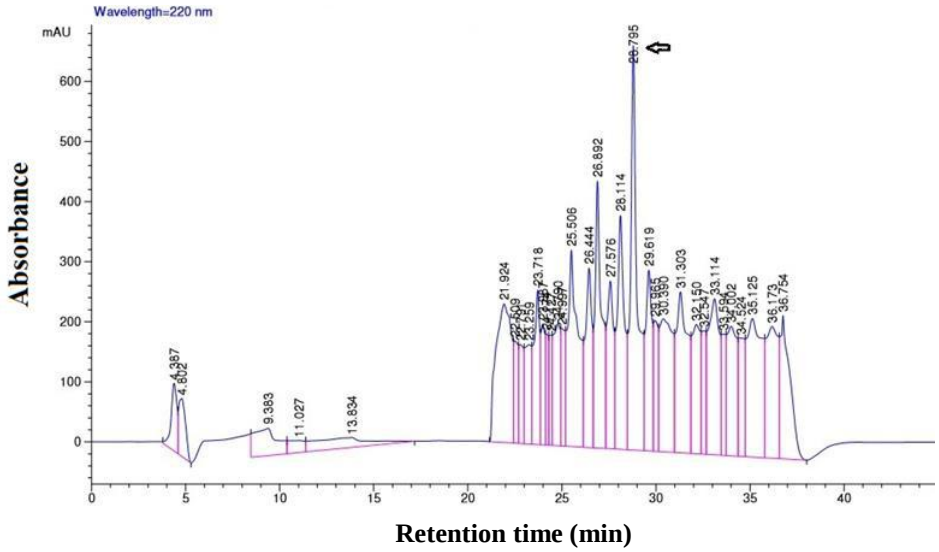

Fig 7: HPLC chromatogram of sample exposed to Moringa oleifera seed solution. Profenofos (28.795 min) is identified.

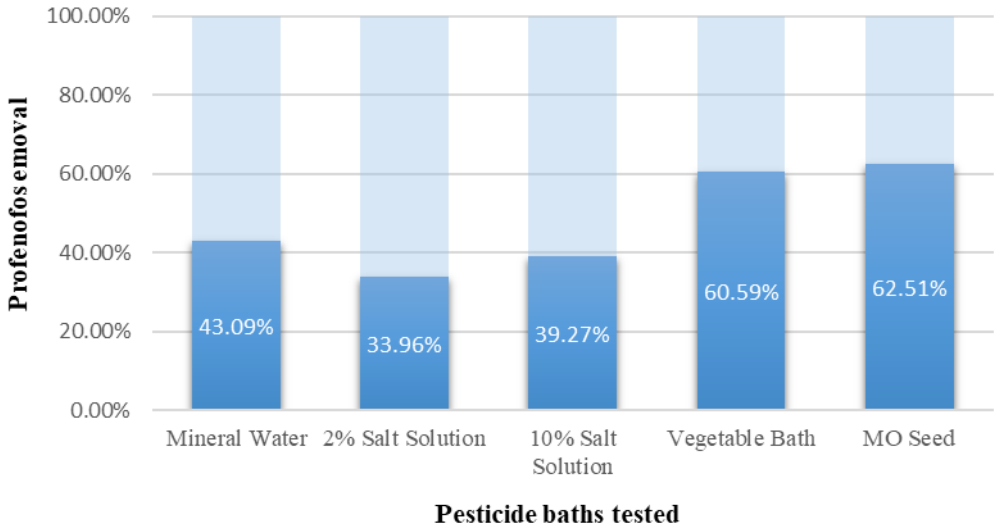

Fig 8: Percentage removal results of the various pesticide remediation baths.

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The decontamination efficiency results in this study are distinctly lower to the findings found amongst related publications. Such articles routinely focus on data hinged on food crops achieving pesticide concentrations below their MRLs with respect to time. When this research topic was being drafted, its essence was never on attempting to achieve MRLs onto the potatoes, but to analyze and review the decontamination strengths of each pesticide bath exclusively. The European Commission declared the MRL for potatoes exposed to profenofos to be 0.01 mg/kg, while the concentration of profenofos used in this study was 500 mg/kg. This amount lies between 298 to 700 mg/kg, thus qualifying it as a lethal dose (LD50); based on laboratory animal oral-method acute toxicity exposure (FAO, 2007). Such an amount makes this study unique in that it allows the bath types to be tested to an extreme magnitude within such a limited timeframe; with the compressed time duration focused on minimizing half-life opportunities. Indeed, because this study used vastly larger concentrations of pesticide compared to other articles, the high reduction percentage would likely not be achievable. Furthermore, an added benefit to the large concentration helped to simplify the HPLC analysis due to the chromatograms peaks for profenofos being unable to be mistaken from any other analyte.

In this study, the *Moringa oleifera* seed was determined as the most effective decontamination method for profenofos, immediately followed by a vegetable bath wash; possessing only a 1.92% difference in effectiveness. Both salt solutions exhibited poorer removal percentages than the simple mineral water rinse alone. While these results are contradictive to the findings conducted by Rao *et al*., (2014), similar results were observed Shalaby (2016) and Shiboob (2012). Although the *Moringa oleifera* seed performed only marginally better than the vegetable bath, what makes the difference profound is when cost comparisons between the two methods are also considered. At the time of this experiment, performing the vegetable bath could be made at a cost of Rp. 5,334 IDR ($0.40 USD)/500 mL bath whereas the *Moringa oleifera* seed bath could be made at a cost of Rp. 1,000 IDR ($0.07 USD)/ 500 mL bath. In addition, no alteration in potato-taste was proclaimed from participants during the decontamination bath taste experiment.

IV. CONCLUSION AND RECOMMENDATIONS

This study was an introductory examination to determine the potential capability of the *Moringa oleifera* seed as an effective pesticide removal apparatus. The ability of the seed to remove high concentrations of profenofos within a short timeframe has been pronounced. It's simple, quick, and low-cost implementation plus lack of food-crop-taste impairment, makes the overall process appealing to the everyday individual.

Future research on this study involves replicating the MO seed procedure to allow quantitative data to be manifested. Other commonly used pesticides need to be subjected to the MO seed bath, with successful results, before the bath can be proclaimed as highly effective. In addition, variances in MO seed dosages when used for pesticide remediation baths need to be investigated.

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