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In vitro Degradation of Profenofos by Rice Grain Rinse Water Lactic Acid Bacteria (LAB)

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ABSTRACT

Widely used in fermentation industry, lactic acid bacteria (LAB) are not only probiotic but also capable of degrading pesticides. We evaluated in the laboratory degradation of profenofos 50% EC by the LAB present in rice grain rinse water cultured in LAB-specific de Mann Rogosa Sharpe (MRS) medium. A single colony of the isolated LAB was inoculated in MRS broth, kept in shaker for 24 h before 10 ml broth was transferred to 25 ml test tube inoculated with profenofos 50% EC at 1 ppm and checked 24, 48 and 72 h after inoculation for its residue by liquid-liquid extraction and gas chromatographic analysis. The results revealed that profenofos concentration decreased to 0.75, 0.44 and 0.25 ppm at 24, 48 and 72h, respectively, indicating the degradation of profenofos by the rice rinse water LAB.

Keywords: *Lactic acid bacteria, Rice grain rinse water, Profenofos degradation*

Introduction

Pesticides are one of the components of integrated pest management in farming. However, farmers often prefer insecticides which contaminate the environment, leaving residues in the food chain detected in products ranging from meat, poultry, fish, vegetable oils, nuts, and various fruits and vegetables (Kuchler *et al.,* 1996; Begum and Patil, 2016). Despite precautions and monitoring means such as crop-specific maximum residue limits (MRLs), pesticide residues are found more than the permissible limits in vegetables (Dureja *et al.,* 2012). Thus there is a need to evolve strategies to pesticide residues in the food chain. Microorganisms are primarily used in bioremediation to degrade environmental contaminants into less toxic forms (Vidali, 200l). Lactic acid bacteria (LAB) are widely used in food processing for fermentation (Faye *et al*., 2012). Described as probiotics, they are live microorganisms that confer a health benet on the host when consumed in appropriate amounts in the food (FAO/WHO, 2001). Being safer to higher animals and widespread on plants and in animal products, they have the potential to dissipate pesticide residues as well. For example, LAB isolated from fermented kimchi were able to degrade chlorpyrifos residues completely in 9 days (Cho *et al.,* 2009). The water after washing rice grains before cooking is a rich source of LAB used in natural farming (Ikeda *et al.,* 2013). The objective of this study was to evaluate the potential of rice grain rinse water LAB in degrading profenofos residues under laboratory conditions.

Materials and Methods

The experiment was conducted in the Toxicology Laboratory (Temp. 35.0 ± 5.1ºC, RH 81.0 ± 3.3%) at

Anbil Dharmalingam Agricultural College and Research Institute, Tiruchirappalli, Tamil Nadu. To collect the LAB from rice grain rinse water, 200 g rice grains were soaked in 400 ml water and the grains were discarded the next day to leave the rinse water undisturbed for LAB multiplication before isolation using the LAB-specific MRS (de Mann Rogosa Sharpe) agar medium (Himedia). The composition of the culture medium comprised *Lactobacillus* MRS Agar (67.15 g), $CaCO_3$ (8.0 g) and distilled water (1000 ml) at pH 6.5. Calcium carbonate ($CaCO_3$) was added (0.8 g/100 ml) to induce better LAB growth (Aween *et al.*, 2012). Cycloheximide (0.1%) was added to the medium before serial dilution and plating in order to prevent the fungal growth. A single colony of LAB was inoculated to the MRS broth and kept in shaker for 24 h. From the above stock culture, 10 ml broth was transferred to 25 ml test tube before inoculating with profenofos 50% EC (Nagarjuna Agrochemicals) at 1 ppm and checked for its residual concentration after 24, 48 and 72 h by gas chromatography (GC) modified from Ghanem *et al.* (2007) by drawing samples (1 ml) from the LAB culture flasks and homogenizing in a shaker for one hour at 150 rpm. Five ml of the homogenized broth was used for residue analysis. To extract the profenofos residue from the LAB broth, 50 ml of dichloromethane and 10 ml of 10 per cent sodium chloride solution were added to the homogenised LAB broth in a separating funnel. The mixture was vigorously shaken for two minutes, releasing the pressure intermittently, and left to stand until phase separation took place. The dichloromethane layer (bottom) was collected in a conical flask by passing through anhydrous sodium sulphate and then the aqueous sample was re-ex-

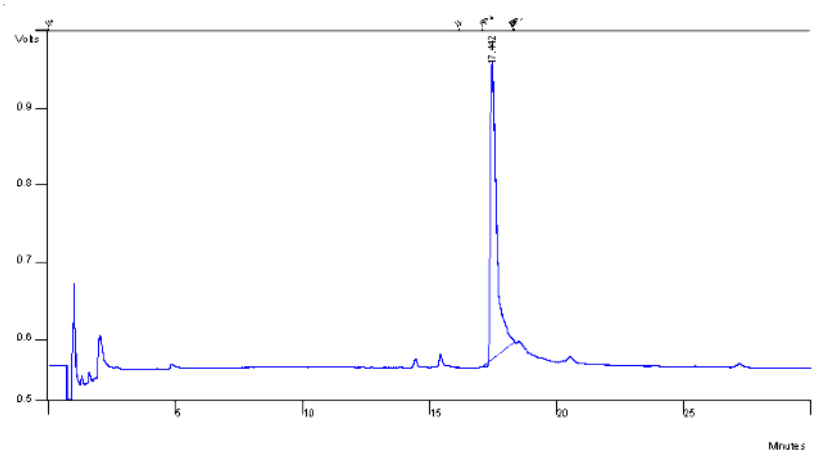


Fig. 1. Profenofos residue 24 h post inoculation detected by GC (RT=17.442)

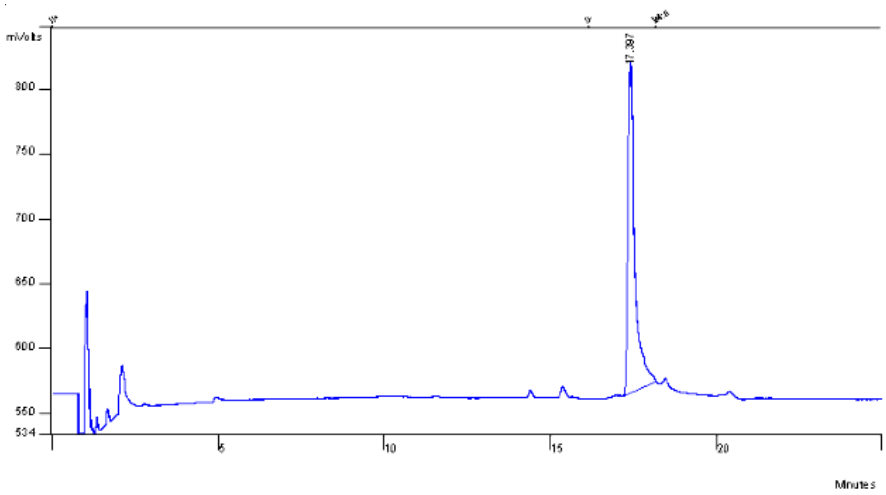


Fig. 2. Profenofos residue 48 h post inoculation detected by GC (RT=17.397)

tracted twice with dichloromethane (25 ml x 2). The organic layer (dichloromethane) was combined and concentrated to 2 ml in a rotary vacuum evaporator at 40 °C. The residue was re-dissolved in 5 ml hexane before injected into GC-ECD operated under the following conditions: Model, GC-VARIAN 3800; Column, Capillary - DB-5, 30 m x 0.25 mm x 0.25 µm; Detector, Electron Capture Detector (ECD); Flow rate, 1.0 ml/min.; Carrier gas, Nitrogen; Sample injection volume; 1 µL; Total run time; 30 min. The amount of insecticide residues recovered was quantified by comparison of the peak area of the standard with that of the unknown sample under identical conditions of operation. The amount of residue recovered in ppm was calculated as follows: Residue (ppm) = As / Astd $_{X}$ Wstd / Ws $_{X}$ Vs / Asj, where, As = Peak area of the sample; Astd = Peak area of the standard; Wstd = Weight of the standard in ng; Ws = Weight of the sample in g; Vs = Volume of the sample (final extract in ml); Asj = Aliquot of the sample injected in µl.

Results and Discussion

The GC results showed that profenofos concentration decreased from 1.0 ppm to 0.75 ppm (Fig. 1), 0.44 ppm (Fig. 2) and 0.25 ppm (Fig. 3) after 24, 48, and 72 h of inoculation, with its retention time confirmed by organophosphorus (OP) mix injected into the GC (Fig. 4). This indicates that the time-dependent dissipation of profenofos concentration was caused by the LAB from the rice grain rinse water. There are several bacteria, fungi and actinomycetes that cause pesticide degradation in nature (Hindumathy and Gayathri, 2013). Ubiquitous and even epiphytic (Harshini *et al*., 2018), LAB are a harmless group of Gram-positive, non-motile, non-respiring, non-spore forming bacteria (cocci or rods)

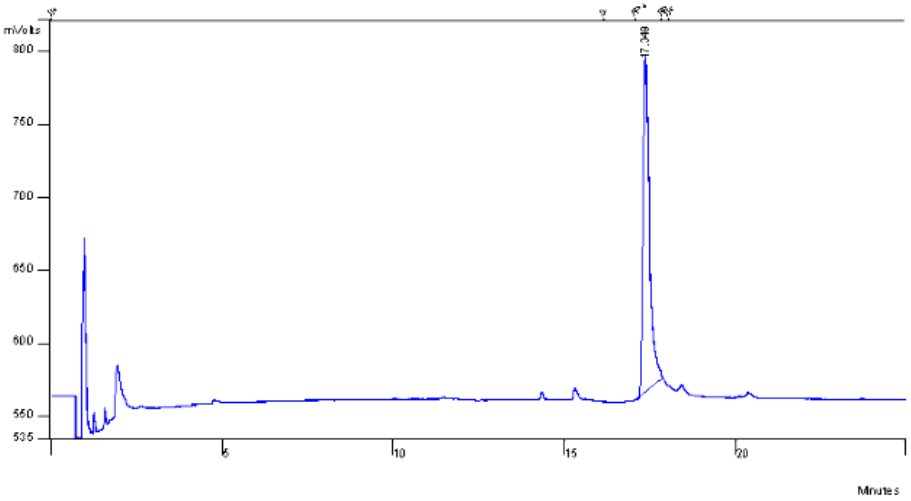
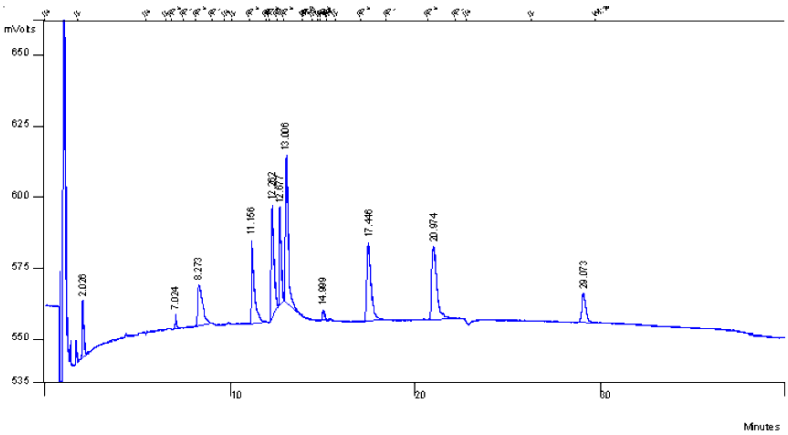


Fig. 3. Profenofos residue 72 h post inoculation detected by GC (RT=17.349)



Peak name	Retention Time (min.)	Area
Acephate	2.026	106440
Phorate	7.024	35469
Dimethoate	8.273	257034
Methyl parathion	11.156	358141
Fenitrothion	12.262	377254
Malathion	12.677	267833
Chlorpyriphos	13.006	506539
Quinalphos	14.999	26346
Profenofos	17.446	409312
Ethion	20.974	481510
Triazophos	29.073	164531

Fig. 4. Standard chromatogram for organophophorus mix.

from the genera *Aerococcus, Cornebacterium, Enterococcus, Lactobacillus, Lactococcus, Leuconostoc, Oenococcus, Pediococcus, Streptococcus, Tetragenococcus, Vagococcus and Weissella* (Klein *et al*., 1998). They occur on rice grains and in rice-based fermented foods (Rhee *et al.,* 2011; Ray *et al*., 2016). Strains of *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus*, and *Weissella* are found in whole-crop paddy rice silage (Ennahar *et al*., 2003). They are capable of degrading pesticide residues too (Patel *et al.,* 2010). This study has also confirmed that the LAB present in rice grains could reduce pesticide residues. In dairy products lactic acid fermentation has been proved to reduce the level of organophosphorus pesticides (Bo and Zhao, 2010). Zhou and Zhao (2014) found such LAB species present in milk as *Lactobacillus casei, L. bulgaricus, L. rhamnosus, L. acidophilus* and *Streptococcus thermophilus* to degrade organophosphorus insecticides, namely, chlorpyriphos, chlorpyriphos-methyl, diazinon, dichlorvos, fenthion, malathion, phorate, pirimiphos-methyl and trichlorphon. Phosphatase produced by LAB is considered as one of the factors responsible for degradation of organophosphorus pesticides (Zhang *et al*., 2014). It may be concluded that LAB found in food grains like rice not only help in food processing by fermentation (Tohno *et al*., 2013) but also in food safety as observed in this investigation by causing pesticide residues to dissipate as they enhance food safety by producing antimicrobial factors such as lactic acid, bacteriocins, CO_2, H_2O_2 and ethanol, which facilitate inhibition or elimination of food-borne pathogens as well as by destroying undesirable toxic components (Holzapfel *et al*., 2002; Magala *et al.,* 2015). Thus there is a potential for making home-made LAB formulations that can be even mixed with pesticide spray fluids at the time of spraying to reduce harmful pesticide residues in the environment and food.

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