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# Effects of Mangosteen's Peels and Rambutan's Seeds on Toxicity, Esterase and Glutathione-S-transferase in Rice Weevil (*Sitophilus oryzae* L.)

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## ABSTRACTS

Peel of Mangosteen (*Garcina mangostana* L.) and seed of rambutan (*Nephilium lappaceum* L.) were attracted by Soxhlet's extraction using ethanol as a solvent. Both extracts were trialed with adult rice weevils (*Sitophilus oryzae* L.) by the impregnated filter paper method for the peel of mangosteen extracts, weevils showed $LC_{50}$ at 24 hours at 4.91 ± 1.19%w/v. Seed of rambutan extracts showed $LC_{50}$ at 6.81 ± 1.04%w/v.The addition of synergists, PB, TPP and DEM in the extracts reduced overall $LC_{50}$ showing SR of ca. 1.3-1.6 folds. The *in vitro* enzyme studies in lived rice weevil after be tested in both extract at 12 hours showed mangosteen's peel extracts inhibited esterase and glutathione-S-transferase (GST) activities ca. 1.6 time. Rambutan's seed extracts reduced esterase and GST activities ca. 1.33- 1.63 fold. The addition of synergists decreased activities of these enzymes more than 50% showing of CF ca. 1.19-12.17. This result suggested that synergists in the extracts could increase efficiency for the control of rice weevils.


**Key words:** mangosteen extracts, rambutan extracts, rice weevils, detoxification enzymes

## INTRODUCTION

Thailand is one of the rice exporting countries in the world. Most of Thailand farmer income comes from the sale of rice products. Also seventy percent of Thai export good is rice which making more than the value of 70,000 million bath a year (Agro-economic, 2001). Thai farmers applied a lot of insecticides in the rice field and in the storage for more than five decades (Bunsit, 1993) to kill many insect pests mostly rice weevils (*Sitophillus oryzae*). We do not want contaminated rice products as well as any resistant insects in the rice. On one hand, synthetic insecticides are dangerous to both human beings and our environment. They induce health problems, are harmful to non target organisms, pollute water, soil, air and our agricultural products, and also create insecticide resistant insects. On the other hand, plant extracts do not possess the problems mentioned above because they are biodegradable (Visetson *et. al*, 2002), giving no toxic effect to human beings (Udomchoak, 1985), fish and bees (Visetson, 2001). The *Sitophilus oryzae* L. is also the key pests to most of the agricultural products, rice, corn, beans, oats and rye damaging up to the 70% of the agricultural products per year (Visetson, 1991). A recent report also showed that this kind of


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insect had been developing insecticide resistance for up to 4 fold (Soderlund and Bloomquist, 1990). Thailand has been increasing the import of synthetic insecticides to control this insect for years making the rice products unsafe for consumption as well as insect resistance.

The successful researches on plant extracts against some key agricultural pest have been developing by many scientists. Most of them have shown the effectiveness in terms of $LC_{50}$ against many key pests in Thailand. A lot of insecticidal plants namely, neem (*Azadirachta indica* L.) (Visetson, 2001), derris (*Derris elliptica* L.) (Visetson *et al*., 2001), lemon grass (*Cymbopogon winterinus* Jewitti) (Thummasarangkoon, 2000), galunga (*Alpinia galunga* L.) (Wattanasombat, 1995), siam weeds (*Chromolaena odonata* L.) (Saekin, 2000) and nutgrass tuber (*Cyperus rotandus* L.) (Ruamthum, 2002) have revealed good tendency for insect control. This research uses mangosteen's peel and the seed of rambutan against the adults *Sitophilus oryzae* L. showing the control efficiency in terms of $LC_{50}$. Piperonylbutoxide, triphenylphosphate and diethylmaleate were used as synergists. Moreover, the general esterase and glutathione-S-transferase enzymes were trialed using enzyme-substrate assays with spectrophotometer to reveal its detoxification mechanisms. These mechanisms introduced us for increase control efficiency by the addition of synergists into the extracts.

## MATERIAL AND METHOD

The *Sitophilus oryzae* L. were received from the Division of Entomology and Zoology, Department of Agriculture, Bangkok. The Completely Randomized Design with 5 replicates of 60 sampling *Sitophilus oryzae* L. unit each replicate. In the synergist experiments, 10%w/v of piperonyl butoxide (PBO), triphenyl phosphate (TPP) and diethylmaleate (DEM) were added to the concentrations prior to use. The peel of mangosteen extracts and the seed of rambutan extracts were modified from the method of Visetson (1991, 2001) by Soxhlet's extraction with 95% ethanol at 75° C for 8 hours. The evaluation of the active compounds, mangostin and saponin followed the methods of Schmutterer (1990). The crude extracts were passed through a column packing with alumina oxide, salicilic acid and anhydrous sodiumsulfate trisodiumcitrate to evaluate of active compounds. The mangostin from peels of mangosteen and saponin from seeds of rambutan were quantified by HPLC-UV detector, modified from Feuerhake and Schmutterer (1982). Impregnated filter paper method modified from Visetson (1991) was used to evaluate their $LC_{50}$ and the actual mortality percentages were adjusted by using Abbott's formula (Matsumura, 1976). The esterase and glutathione S-transferase activity were trialed using the modified method of Visetson *et al*. (2002) using paranitrophenol and chlorodinitrobenzene as substrates. The products were quantified by UV-spectrophotometer at 400 and 344 nm, respectively.

## RESULT AND DISCUSIION

### 1. Yield of extraction and active compounds from plant materials

The yields of extracts showed 29.46 ± 3.12 and 27.77 ± 2.54% w/w for mangosteen and rambutan, respectively (Table 1). The amount was higher than the yield from neem (*Azadirachta indica*) seed kernel extracts which showed ca. 20.12% w/w (Visetson, 2001) but giving more or less the same as crude derris extract which showed ca. 26.12% w/w (Visetson and Milne, 2001). Although all of plant materials were extracted by the same method, solvent and the same temperature, the yields of the extracts are usually different. This may be due to the difference in plant compositions such as tannin, cellulose, chlorophyll, protein and lipid in the materials. The mangostin from peels of

**Table 1** Yields from Soxhlet's extraction and active compounds in the products of the two plant materials, mangosteen peel *(Garcina mangostana* L.) and rambutan seed (*Nephelium Lappaceum* L).

| Plant materials | % yield (w/w) [1, 2] | %active compound (w/w) [1, 3] |
|---|---|---|
| Mangosteen peel | 29.46 ± 3.12[a] | 2.58 ± 2.11[a] |
| Rambutan seed | 27.77 ± 2.54[b] | 5.41 ± 3.12[b] |

1 means followed by different letters within the same column are significantly different at $P < 0.05$, DMRT

2 means ±SD, 5 replicates, using Soxhlet's extraction method as mentioned in the text.

3 active compounds; mangostin for mangosteen, saponin for rambutan were quantified using HPLC-UV detector, modified from Feuerhake and Schmutterer (1982).

mangosteen and saponin from seeds of rambutan after partial purification mentioned in the text were applied on to HPLC-UV detector. The active compounds revealed 2.58 ± 2.11 and 5.4 ± 3.12% w/w, respectively comparing with the standards (Table 1). These amounts were higher than the amount of azadirachtin from neem seed kernel extracts which showed ca. 1% w/w (Visetson, 2001) but less than the amount of rotenone from derris roots which revealed ca. 6.36% w/w (Visetson and Milne, 2001). However, the active compounds extracted from these plants were similar in quantity compared to the selinadiene from nut grass tubers (Visetson *et al*., 2001).

**2. Toxicity testing with or without synergists using an impregnated filter paper method**

Using the impregnated filter paper method with varying extract concentrations against *Sitophilus oryzae* L., $LC_{50}$ values for both types of plant extracts were 5.5 ± 1.17 for mangosteen and 7.35 ± 1.11% w/v for rambutan extracts, at 12 hours exposure (Table 2). The longer exposure time at 24 hours did not give any more significant difference on the $LC_{50}$. The addition of 10% TPP, DEM, and PBO resulted in synergist ratios (SR) of 1.33 to 1.63 fold for all experiments. This result indicated that TPP, DEM or PBO could be mixed to increase extracts efficiency. These results were similar to those of Visetson and Milne (2001) who found that addition of PB and DEM to rotenone from derris extracts increased rotenone efficiency by 2 folds. The correlation between concentration and mortality in most experiments indicated $r^2$ of 0.85 – 0.98 showing that the effects of active compounds on the mortality of weevils were highly correlated. This result was similar to that of Visetson (1991) who worked with these synergists added to cyfluthrin in the control of *Tribolium castaneum* and Visetson (2001) who used TPP and PB in neem seed kernels extracts in the control of *Callosobruchus maculatus*. Although there was different in organism tested, all beetles mentioned above were the stored product insects which have food over lapping behavior. Thus, we could assume in some ways that these two plant extracts could be used as an insecticide alternative.

**3. Enzyme activity**

Both types of extracts alone gave less decreased esterase and glutathione- S-transferase activities showing of CF from 1.19 – 2.59 fold compared to untreated control (Table 3). Decreased esterase and glutathione- S-transferase levels after application of plant extracts had been reported in a number of research works (Wattanasombat, 1995; Thummasarangkoon, 2000; Ruamthum, 2002). Furthermore, the addition of TPP and DEM showed dramatically increased CF of 11.90 and 12.17 against mangosteen, respectively. However, with

**Table 2** $LC_{50}$ values (%w/v) of *Sitophilus oryzae* L. affected by crude extracts of mangosteen peel and rambutan seeds with or without synergist after 12 and 24 hrs after exposure using an impregnated filter paper method.

| Type of extract | Hours | Mangosteen[1,2] | $r^2$ | [SR ][4] | Rambutan[1,2] | $r^2$ |
|---|---|---|---|---|---|---|
| None[3] | 12 | $5.50 \pm 1.17^{b}$ | 0.99 | | $7.35 \pm 1.11^{b}$ | 0.98 |
| | 24 | $4.91 \pm 1.19^{ab}$ | 0.98 | | $6.81 \pm 1.04^{ab}$ | 0.91 |
| + TPP | 12 | $4.13 \pm 1.13^{ab}$ | 0.89 | | $5.30 \pm 1.10^{ab}$ | 0.96 |
| | | [1.33] | | | [1.39] | |
| | 24 | $3.64 \pm 1.12^{a}$ | 0.84 | | $4.22 \pm 1.13^{a}$ | 0.93 |
| | | [1.35] | | | [1.61] | |
| + DEM | 12 | $4.13 \pm 1.08^{ab}$ | 0.88 | | $5.45 \pm 1.19^{ab}$ | 0.99 |
| | | [1.33] | | | [1.34] | |
| | 24 | $3.69 \pm 1.08^{a}$ | 0.85 | | $4.43 \pm 1.12^{a}$ | 0.95 |
| | | [1.33] | | | [1.54] | |
| + PBO | 12 | $3.99 \pm 1.13^{a}$ | 0.92 | | $4.77 \pm 1.18^{a}$ | 0.97 |
| | | [1.38] | | | [1.54] | |
| | 24 | $3.67 \pm 1.14^{a}$ | 0.88 | | $4.18 \pm 1.10^{a}$ | 0.94 |
| | | [1.34] | | | [1.63] | |

1 means followed by different letters within the same column are significantly different at $P < 0.05$

2 means $LC_{50}$ values ±SD, 5 replicates, 60 individual /replicate, 12 and 24 hours check per batch from F2-generation for all experiments.

3 "None" means no synergist was added to the various concentrations.

4 SR = ($LC_{50}$ none)/ ($LC_{50}$ with synergist) while $r^2$ was a correlation determination between concentration and mortality.

the rambutan seed extracts the addition of both synergists did not give much CF (ca. 5 –6 fold). The increased CF by addition synergists in other plant extracts was confirmed by Visetson *et al*. (2002) who had done with synergists in the nut grass tuber extracts showing increased efficiency in terms of $LC_{50}$ against the golden apple snails. On the other hand, glutathione -S- transferase activity did not change much in CF values when TPP, DEM or PBO was added to both kinds of extracts. This is an indication of complete metabolisms of both saponin and mangostin in phase I reaction possibly hydrolysis by esterase. However, more works should be done on purified enzymes using different substrates before final evaluation have been made.

## CONCLUSION

Peel of mangosteen (*Garcina mangostana* L.) and seed of rambutan(*Nephilium lappaceum* L.) were extracted by Soxhlet's extraction using ethanol as a solvent showed good control *Sitophilus Oryzae* L. which indicated $LC_{50}$ value at 24 hours ca. 4.91%w/v. The addition of synergists in both extracts, reduced overall $LC_{50}$ showing SR of 1.33- 1.63 folds. The *in vitro* study indicated that both kinds of extracts inhibited esterase and glutathione-S-transferase activities up to 1.19 folds. The addition of synergists decreased activities of this enzyme more than 50%. These results showed that both extracts can be used as insecticide alternatives for rice weevils control and the use of synergists could increase the control efficiency.

**Table 3** Esterase and glutathione-S- transferase (GST) activities of adult *Sitophilus oryzae* L. against both extracts after 12 hours exposure.

| Type of extract | Mangosteen | | Rambutan | |
|---|---|---|---|---|
| | Esterase[1,2,3] | GST [1,2,3] | Esterase [1,2,3] | GST [1,2,3] |
| | [CF] [4] | | | |
| Control | 26.54 ± 1.03[b] | 8.18 ± 2.13[b] | - | - |
| None[5] | 22.14 ± 8.03[b] | 5.26 ± 1.09[a] | 20.38 ± 1.05[b] | 3.15 ± 2.11[a] |
| | [1.19] | [1.56] | [1.30] | [2.59] |
| + TPP | 2.23 ± 1.03[a] | 5.11 ± 1.12[a] | 5.20 ± 1.01[a] | 3.11 ± 1.01[a] |
| | [11.90] | [1.60] | [5.10] | [2.63] |
| + DEM | 2.18 ± 1.03[a] | 4.98 ± 1.29[a] | 4.12 ± 1.00[a] | 4.11 ± 1.01[a] |
| | [12.17] | [1.64] | [6.44] | [1.99] |
| +PBO | 22.22 ± 1.03[b] | 4.23 ± 1.01[a] | 5.18 ± 1.12[a] | 3.19 ± 1.03[a] |
| | [1.19] | [1.93] | [5.12] | [2.56] |

1 means followed by different letters within the same column are significantly different at $P < 0.05$, DMRT

2 means activities ±SD, 5 replicates, n= 30 adults were employed, 12 hour check per batch from F2-generation for all experiments.

3 enzyme assays were followed Visetson and Milne (2001), the unit of esterase and glutathion-S-transferase are nM paranitrophenol produced/min/mg protein and nM CDNB conjugated product/min/mg protein.

4 CF is a correlation factor = (enzyme activity of control)/ (enzyme activity of treatment).

5 ." None" means no synergist was added to the concentrations while control means spraying with water onto the weevils.

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