ABSTRACT

The plant extract; KU Natural Extocide® was stored at room temperature (19.5 – 39 °C) for up to 24 and 30 months. *In vitro* acaricidal activities bioassay of three different concentrations (high, medium and low concentrations) of the 24 and 30 months storage products were performed on eggs, unfed larvae, adult male and engorged female brown dog ticks (*Rhipicephalus sanguineus*). All of three concentrations of 24 and 30 months storage products exhibited 100% anti-hatching activity of the eggs. The mortality of unfed larvae and adult male ticks were 93-100% and 87-99% at 2 h after spraying with those three concentrations of the 24 and 30 months storage products.
products, respectively. However, all of the still survival larvae and male ticks were died at 20 h after spraying. Those of 24 months storage products were killed female ticks 50-61% and 84-100% at 24 h and 7 days after spraying, respectively. The still survival ticks were unable to lay eggs. While the 30 months storage products were killed 41-68% and 82-97% at 24 h and 7 days after spraying, respectively. These still survival female ticks laid a little crispy and non-hatching eggs.

This results suggested that the KU Natural Extocide® might be a commercial herbal acaricide products since its 30 months shelf-life at room temperature is still highly effective against every stage of dog ticks. It might be use to spray in the house where the dog often lying down and the ticks drop down for molting or laying eggs.

Key Words: Plant extracts, KU Natural Extocide®, Brown dog ticks, Long storage
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INTRODUCTION

Recently, herbal products are world-wide interesting for alternative medicine since they are safe and more synergistic active substances in the plant. The herbal insecticides are also interesting for controlling external parasites of domestic animals since the synthetic insecticides leave the toxic residues in environments and many external parasites especially ticks can develop resistant strains, resulting of their delay degradation. Therefore, the original use of plants crude-extracts having acaricidal activity seems to come up with the promising results. Our previous works revealed that there were many plants having high acaricidal activity (Chungsamarnyart et al., 1988, 1990, 1991 a, b; Chungsamarnyart, and Jansawan,1994, 2001). Some of them could be practical use; the crude-extracts of sugar apple seeds (Chungsamarnyart et al., 1991c), the volatile oil from lemon and citronella grasses (Chungsamarnyart and Jiwajinda, 1992; Chungsamarnyart and Jansawan.1992); the yam bean seeds (Chungsamarnyart and Jansawan, 1993); the peel oil of Citrus spp. (Chungsamarnyart and Jansawan, 1996) and the tamarind fruits (Chungsamarnyart and Jansawan, 2001). However, most of plant crude-extracts were short expiry. This KU Natural Exticide® is one of the possible long shelf-life commercial herbal acaricide since the acaricidal activity of the 1, 6, 12 and 18 months storage product was still high activity (Chungsamarnyart, et al, 2003 a, b.). This study is try to find the longest storage periods of the product that it has still high efficacy.
MATERIALS AND METHODS

The KU Natural Extocide® (herbal acaricide) was stored at room temperature (19.5 – 39 °C) for 24 and 30 months. Its acaricidal activity of various concentrations (high, medium and low concentrations) of the 24 and 30 months storage products was in vitro bioassay on eggs, unfed larvae, adult male and engorged female of brown dog ticks (Rhipicephalus sanguineus). For the anti-hatching activity, one milliliter of each concentration of herbal acaricide was tropical applied on eggs of 20 engorged female ticks. Then the eggs were observed the anti-hatching activity at 1 month after application. The acaricidal activity on unfed larvae, adult male and engorged female ticks were tested by tropical spraying the products on them and the excess of the products were cleaned by tissues paper. The mortality of larvae and adult male ticks were observed at 2 and 20 h after spraying. The mortality of engorged female ticks were observed at 24, 48 h and 7 days after spraying (Chungsamarnyart and Jansawan, 1990). The corrected mortality of ticks were calculated by Abbott’s formula (Abbott, 1925). The mean (%) of corrected mortality of adult male and engorged female ticks were average of 5 replications (20 adult male or engorged female ticks per replication), but those of unfed larvae was average of 3 replications (100-600 larvae per replication). The control ticks and eggs were intact ones.

RESULTS

In vitro acaricidal activities of KU Natural Extocide® storage 24 and 30 months at room temperature were bioassayed on eggs, unfed larvae, adult male, and engorged female ticks (Rhipicephalus sanguineus) after spraying with three concentrations (high, medium and low concentrations)

All of three concentrations of the 24 and 30 months storage products exhibited 100% anti-hatching activity. (Table 1).

The larvicidal activity at 2 h after spraying, three concentrations of 24 months storage products showed 100%, 100% and 98.73% dead of the larvae, respectively. While the 30 months storage product killed the larvae 99.76%, 99.25% and 99.54%, respectively (Table 1). The all still survival larvae were died at 20 h after spraying.

The acaricidal activity on adult male ticks at 2 h after spraying, three concentrations of the 24 months storage products showed 100%, 100% and 93% dead, respectively. While the 30 months storage product showed 89%, 89% and 87% dead, respectively (Table 1). However, all still survival male ticks were dead at 20 h after spraying.
The acaricidal activity on engorged female ticks of the 24 months storage products with those three concentrations showed 61%; 56%, and 50% dead at 24 h after spraying, respectively. While the 30 months storage products began much more variation and the ticks were died 68%, 59% and 41% at 24 h after spraying, respectively (Table 2.).

At 7 day after spraying, the ticks were died 100%, 100% and 84% after spraying with those three concentrations of 24 months storage product, respectively. While those of 30 month storage product showed 97%, 94% and 82% dead of ticks, respectively (Table 2.).

At 7 day after spraying with 24 months storage product, all still survival ticks could not laying. While those three concentrations of 30 months product, the still survival ticks laid a little, crispy and non-hatching eggs, 2%, 6% and 3%, respectively (Table 2.).

Table 1. Acaricidal activity of KU Natural Extocide® on eggs, larvae and male brown dog ticks

<table>
<thead>
<tr>
<th>Storage periods (mth)</th>
<th>Conc. of KU Natural Extocide®</th>
<th>Anti-hatching of eggs (%)</th>
<th>Unfed larval Mortality (Mean, %) after spraying</th>
<th>Male tick Mortality (Mean, %) after spraying</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>2 h</td>
<td>20 h</td>
</tr>
<tr>
<td>24</td>
<td>High</td>
<td>100.00</td>
<td>100.00</td>
<td>-</td>
</tr>
<tr>
<td>24</td>
<td>Medium</td>
<td>100.00</td>
<td>100.00</td>
<td>-</td>
</tr>
<tr>
<td>24</td>
<td>Low</td>
<td>100.00</td>
<td>98.73 ± 1.71</td>
<td>100.00</td>
</tr>
<tr>
<td>30</td>
<td>High</td>
<td>100.00</td>
<td>99.76 ± 0.40</td>
<td>100.00</td>
</tr>
<tr>
<td>30</td>
<td>Medium</td>
<td>100.00</td>
<td>99.25 ± 1.29</td>
<td>100.00</td>
</tr>
<tr>
<td>30</td>
<td>Low</td>
<td>100.00</td>
<td>95.54 ± 2.62</td>
<td>100.00</td>
</tr>
</tbody>
</table>

Table 2. Acaricidal activity of KU Natural Extocide® on engorged female ticks

<table>
<thead>
<tr>
<th>Storage periods (mth)</th>
<th>Conc. of KU Natural Extocide®</th>
<th>Corrected Mortality of engorged female ticks (Mean, %) after spraying</th>
<th>Corrected (Mean, %) of ticks laying a little, crispy non-hatching eggs after spraying 7 d</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>24 h</td>
<td>48 h</td>
</tr>
<tr>
<td>24</td>
<td>High</td>
<td>61 ± 4.18</td>
<td>63 ± 2.73</td>
</tr>
<tr>
<td>24</td>
<td>Medium</td>
<td>56 ± 7.41</td>
<td>62 ± 7.58</td>
</tr>
<tr>
<td>24</td>
<td>Low</td>
<td>50 ± 5.00</td>
<td>60 ± 6.12</td>
</tr>
<tr>
<td>30</td>
<td>High</td>
<td>68 ± 16.04</td>
<td>70 ± 14.57</td>
</tr>
<tr>
<td>30</td>
<td>Medium</td>
<td>59 ± 17.81</td>
<td>66 ± 10.84</td>
</tr>
<tr>
<td>30</td>
<td>Low</td>
<td>41 ± 16.35</td>
<td>59 ± 21.35</td>
</tr>
</tbody>
</table>
**In vivo** bioassay, the low concentration of 30 month storage product were tested on a few dogs having ticks, flea or lice. The male ticks, flea and lice were came out and fell down within 30 min and they were died within 20 h after spraying. The all most of female ticks were died on the dogs after spraying 24 h.

**DISCUSSION**

The unfed larvae and adult male ticks were killed rapidly within 2 h after spraying with KU Natural Extoicide and the mortality were rapidly effective and very small variability. These results are correspondingly activity with the high efficacy of volatile oil from the lemon and citronella grasses (Chungsamarnyart and Jiwajinda, 1992); the yam bean seeds (Chungsamarnyart and Jansawan, 1993); and the peel oil of *Citrus* spp. (Chungsamarnyart and Jansawan, 1996 a).

The acaricidal acticity of the 24 months storage product on unfed larvae and adult male ticks were still high at 2 h after spraying and a little decrease activity of the 30 months storage product (Table 1). The acute acaricidal activity on engorged female ticks at 24 h after spraying were also a little gradual decrease activity comparing to the previous results of the product storage 1, 6, 12 and 18 months (Chungsamarnyart, *et al*., 2003 a, b.). The activity were much more variation in the 30 months storage product (Table 2). These results showed that the acute acaricidal activity of this product was began to decrease in the 30 months storage product.

The delayed acaricidal activity at 7 days after spraying also began to decrease in 30 months storage product since the mortality of engorged female ticks were 97% and 94% after spraying with the high and medium concentrations of the 30 months storage product, respectively (Table 2). These mortality of engorged female ticks were not 100% as those of the 24 months storage product. However, these delayed acaricidal activity were much lesser decreased than the acute activity comparing to the previous results of the product storage 1, 6, 12 and 18 months (Chungsamarnyart, *et al*., 2003 a, b.). This might be indicated that the acute acaricidal activity were more rapidly decreased than the delayed acaricidal activity of the room temperature storage product.

The anti-laying activity of engorged female ticks was also began to decrease at 30 months storage product since the survival ticks (2-6%) (Table 2) at 7 days after spraying could lay a small number of eggs, but these eggs were crispy and non-hatching eggs (Table 2). Therefore, the 30 months storage product could inhibit the laying and hatching activity in the life cycle of ticks.

The ticks using in this bioassay were collected from Kanchanaburi Animal Shelter. This shelter has been used many synthetic insecticides to control ticks for a long time, but this shelter
still have the ticks problems. It is possible that those ticks might be the resistant strains against many synthetic insecticides. However, the 24 and 30 months storage of the KU Natural Extocide® were exhibited high efficacy on their eggs, larvae, adult male and female stages of those ticks. It might be confirm that this KU Natural Extocide® can be control the insecticidal resistant strain of ticks. Unfortunately, the in vivo bioassay were done only a few dogs. The further study will be test on more many dogs for in vivo evaluation.

ACKNOWLEDGEMENTS

The researchers wish to thank Miss Pimoloor Aagsvothai, the Manager of Kanchanaburi Animal Shelter for her kind providing ticks for this experiment, Miss Charoenchit Thapanathorn for her kind collecting and carrying ticks from Kanchanaburi Animal Shelter and the Director of Nakhon Pathom Meteorological Station for the climate records service. The authors are gratefully acknowledged the Public-Private Technology Development and Transfer Center of Kasetsart University for the grant support.

REFERENCES


