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Radio Frequency Heat Treatment for Controlling Cigarette Beetle in Dried Tobacco

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Abstract

Tobacco (*Nicotiana tabacum* L.) is one of many agricultural commodities produced in Thailand. There are Virginia (flue-cured tobacco) and Burley (air-cured tobacco) typesand Cigarette beetle, *Lasioderma sericorne* F. is the most important insect pest that attacks dried tobacco. The efficacy of radio frequency (RF) heat treatment at 27.12 MHz was examined to control cigarette beetle on dried tobacco. Various growth stages of cigarette beetle were prepared within samples of dried tobacco and were exposed to RF at 55, 60, and 65 °C for 1, 2 and 3 minutes. The results showed that pupal and adult stages of cigarette beetle were the most tolerant stages to RF heat treatments. The RF treatment at 65 °C for 3 minutes is able to cause complete mortality of egg, larval, pupal and adult stages of cigarette beetle4.

Keywords: dried tobacco, *Lasioderma serricorne,* tolerant stage, heat treatment

Introduction

Virginia and Burley tobacco production in Thailand is found in 10 provinces mostly in the north and northeast of Thailand covering about 21,000 ha and is valued at approximately 2 billion US dollars in 2016-2017 cigarette sales. In Virginia and Burley tobacco storage, cigarette beetle, *Lasioderma serricorne* (F.) (Coleoptera: Anobiidae) is the most damaging insect pests of dried tobacco and grain products such as corn, beans and dried herbs in Thailand. Insecticides have been used as the main control measure for managing stored tobacco pests. To avoid chemical contamination in the commodity other control measures such as storage sanitation and pest exclusion to remove sources of infestation should be considered. Several studies have been performed using radio frequency (RF) energy to control stored product insects. Mitcham et al. (2004) tested RF energy to control Codling moth (*Cydia pomonella),* navel orangeworm (*Amyelois transitella*, and Indianmeal moth (*Plodia interpunctella),* at 55°C for 5 minutes and was able to gain complete control.. Lagunas-Solar et al. (2007) also reported that using RF at, 10 kHz to 1050 MHz resulted in >99% mortality of Anguomois grain moth (*Sitotroga cerealella*).

The use of radio frequency, as a heat treatment, has been investigated to control many kinds of insect pest associated with agricultural products (Nelson, 1996). Experiments have been conducted to control many stored product insects to determine the tolerant stages of insects to the RF heat (Table 1.). For heat treatment with radio frequency, commodities are allowed to increase temperature rapidly due to the vibrationof water molecules. Nutapong (2012) tested the efficacy of RF to kill cigarette beetle on packaged dried tobacco which was infested with cigarette beetles in all stages. The results showed that the adult stage was the most tolerance to RF heat treatment at higher temperatures (104 °C) for complete control Radio frequency also has less effect on various kinds of grains compared with conventional heat (Srikam et al., 2014; Wangsapa et al. 2016; Zhou et al. 2015). There is very little research work of RF treatment on dried tobacco. Therefore, this experiment is aimed to examine the reduction of temperatures that causes mortality of cigarette beetle with RF to save energy costs and to reduce the effect on commodity quality.

Table 1. The exposure time of radio frequency heat on insect pests associated with various agricultural commodities which resulted for 100% morality of tolerant stages. (The Pilot scale of 27.12 MHz radio frequency was provided.)

Commodities	Insect pests	Tolerance stages	Temp (°C)	Time exposure	References
Corn	*Sitophilus zeamais*	Adult	92	4 min	Faikrajaipuen et al. (2011)
Feed	*Tribolium casteneum*	Pupa	70	1 min	Bualoi (2009)
Milled rice	*Corcyra cephalonica**	Egg	60	3 min	Luechai (2008)
Milled rice	*Orhyzaephilus surinamensis*	Adult	70	2 min	Srikam et al. (2014)
Milled rice	*Rhyzopertha dominica*	N/A	70	3 min	Janhang (2005)
Milled rice	*Rhyzopertha dominica*	Adult	70	2 min 30 sec	Sumetha et al. (2009)
Mung bean	*Callosobruchus maculatus**	Egg, larva, pupa	74	3 min 40 sec	Na Pijit et al. (2011)
Rough rice	*Sitotroga cerealella**	Egg, pupa	50	3 min 40 sec	Buapud et al.(2012)
Rough rice	*Sitophilus oryzae*	Adult, pupa	65	2 min	Wangsapa et al.(2015)
Tobacco	*Lasioderma sericorne*	Adult	104	4 min	Nutapong (2012)

* Adult was not in the test
N/A =not available

Materials and Methods

Insect rearing and efficacy test of RF treatment to control cigarette beetle

Cigarette beetles were cultured with green leaf tobacco or dried tobacco in flue cured Virginia tobacco (bright tobacco) at 28-32°C with 75% rh in saturated salt solution box in Stored Product Laboratory, at Department of Entomology, Faculty of Agriculture, Chiang Mai University, Chiang Mai, Thailand. Cigarette beetle eggs at 2-4 days old while larvae, pupae, and adults were 20-25, 30-32, 35-40 days old respectively after oviposition were placed on Virginia tobacco at approximately 12% mc. Fifty insects were put in dried tobacco in small plastic cups and tempered to 75% rh to acclimate insects The cup was put in an arena test chamber that is a cylinder of 16 cm in diameter with 5 cm height and was used to be exposed to RF heat t. Pilot scale radio frequency at 27.12 MHz was used to treat all stages of cigarette beetle, at temperatures of 55, 60, 65 °C and for 0, 1,2, and 3 minutes (9 combination treatments). The pilot scale of radio-frequency unit was developed and built from the Institute of Agricultural Engineering, University of Göttingen, Germany.

The effect of RF to cause insect mortality was examined after 42, 22, 12, and 3 days on egg, larval, pupal and adult stages, respectively. The number of survivalors was calculated for the insect mortality by deduction from the total. In the untreated control, insects were not treated with RF but maintained in tobacco leaves as were those exposed to treatments. Each treatment was replicated 4 times with 50 insects per replication. Insect mortality in untreated controls was corrected using Abbott formula (Abbott, 1925). All combinations of treatments between temperatures and time exposure were calculatedusing analysis of variance and LSD test.

Results

Eggs of cigarette beetle showed 79.8% mortality at the treatment using 55°C for 1 minute. The experiment showed complete mortality using RF treatments at 65°C for 1, 2 and 3 minutes. Although in each experimental unit was run individually the longer exposure times also showed serially increasing mortality. The mortality of eggs exposed at 65°C in 1, 2 and 3 minutes showed significantly differences from others at 55 and 60°C (Table 2.). Both temperatures alone and time exposure alone caused significantly difference of insect mortality.

Table 2. Mortality of eggs of cigarette beetle exposed to different temperatures from radio frequency at 27.12 MHz for 1,2 and 3 minutes.

Temperature from RF treatment (°C)	Average mortality of eggs			Total[2/]
	1 minute[1/]	2 minutes	3 minutes	
55	79.79 ± 2.76 c	86.33 ± 3.33 bc	90.65 ± 3.13 b	85.59± 3.16 Y
60	80.00 ± 3.87 c	88.41 ± 2.69 b	93.04 ± 1.97 b	87.15± 3.82 Y
65	100.00 ± 0.00 a	100.00 ± 0.00 a	100.00 ± 0.00 a	100.00 ± 0.00 X
Total[3/]	86.60 ± 6.70 B	91.58 ± 4.25 A	94.56 ± 2.80 A	

[1/]Means in the same column followed by the same letter are not significantly different at P≥0.05 (LSD=6.73).
[2/]Means in the same column followed by the same letter are not significantly different at P≥0.05 (LSD = 3.88).
[3/]Means in the same row followed by the same letter are not significantly different at P≥0.05 (LSD = 3.89).

At 1 minute exposure time of 55°C- RF heat treatment, the mortality of cigarette beetle found 41.27 % with increasing of exposure time. This heat treatment caused 100 % mortality on larvae for either of 60°C for 3 minutes or 65°C for 2 minutes. There were both showed significant difference exposure time and temperature (Table 3).

Table 3. Mortality of larvae of cigarette beetle exposed to various temperatures from radio frequency at 27.12 MHz for 1, 2 and 3 minutes.

Temperature from RF treatment (°C)	Average mortality of larvae			Total[2/]
	1 minute[1/]	2 minutes	3 minutes	
55	41.27 ± 3.62 e[1/]	46.89 ± 1.95 de	52.29 ± 3.66 cd	46.82 ± 3.18 Z[2/]
60	56.81 ± 2.17 c	93.62 ± 0.95 b	100.00 ± 0.00 a	83.48 ± 13.46 Y
65	90.28 ± 0.78 b	100.00 ± 0.00 a	100.00 ± 0.00 a	96.76 ± 3.24 X
Total[3/]	62.79 ± 14.46 C	80.17 ± 16.74 B	84.10 ± 15.90 A	

[1/]Means in the same column followed by the same letter are not significantly different at P≥0.05 (LSD=5.67).
[2/]Means in the same column followed by the same letter are not significantly different at P≥0.05 (LSD = 3.27).
[3/]Means in the same row followed by the same letter are not significantly different at P≥0.05 (LSD = 3.27).

For pupae stage, the mortality also was checked from the survival of insect after exposing to RF heat treatment. The mortality was present in the same pattern as in larval stage but only one treatment which achieve to kill insects all compared to in larval stage. It means the pupae required higher temperature of RF heat than in larval stage. Only 65°C for 3 minute- treatment showed completely control to pupae of cigarette beetle (Table 4).

Table 4. Mortality of pupae of cigarette beetle exposed to various temperatures from radio frequency at 27.12 MHz for 1, 2 and 3 minutes

Temperature from RF treatment (°C)	Average mortality of pupae			Tota[2/]
	1 minute[1/]	2 minutes	3 minutes	
55	70.19 ± 1.21 g	74.11 ± 1.17 f	91.20 ± 1.47 d	78.50 ± 6.45 Z
60	85.61 ± 1.56 e	89.80 ± 1.31 d	96.44 ± 1.19 bc	90.62 ± 3.15 Y
65	94.72 ± 0.79 c	98.37 ± 0.88 ab	100.00 ± 0.00 a	97.70 ± 1.56 X
Total[3/]	83.51 ± 7.16 C	87.43 ± 7.10 B	95.88 ± 2.56 A	

[1/]Means in the same column followed by the same letter are not significantly different at P≥0.05 (LSD=3.12).
[2/]Means in the same column followed by the same letter are not significantly different at P≥0.05 (LSD = 1.81).
[3/]Means in the same row followed by the same letter are not significantly different at P≥0.05 (LSD = 1.82).

Adult mortality was checked after insect exposed to heat for 3 days. The results were similar to those in pupal stage. Only 65°C for 3 minute- treatment showed completely control to pupae of cigarette beetle (Table 4). There were significant differences among temperature treatments (55, 60 and 65°C).

Table 5. Mortality of adults of cigarette beetle exposed to various temperatures from radio frequency at 27.12 MHz for 1, 2 and 3 minutes

Temperature from RF treatment (°C)	**Average mortality of adults**			**Total**[2/]
	1 minute[1/]	**2 minutes**	**3 minutes**	
55	51.38 ± 2.87 d	81.17 ± 2.78 b	87.16 ± 2.26 b	73.24 ± 11.06 Z
60	69.07 ± 5.79 c	88.26 ± 2.65 b	98.93 ± 0.57 a	85.42 ± 8.74 Y
65	81.09 ± 4.71 b	98.95 ± 0.56 a	100.00 ± 0.00 a	93.35 ± 6.14 X
Total[3/]	67.18 ± 8.63 C	89.46 ± 5.17 B	95.36 ± 4.11 A	

[1/]Means in the same column followed by the same letter are not significantly different at P≥0.05 (LSD=8.66).
[2/]Means in the same column followed by the same letter are not significantly different at P≥0.05 LSD = 5.00).
[3/]Means in the same row followed by the same letter are not significantly different at the P≥0.05 (LSD = 5.00).

4. Discussion

Yu et al. (2011) and Conyers and Collins (2006) reported that eggs of cigarette beetle were the most tolerant to air dry heat and complete mortality of eggs required 50°C for 18 hours. Thus RF heat treatments would be an alternative control measure for solving the problem of using large amounts of energy. Nutapong (2012) demonstrated, larval and egg stages of cigarette beetle were more susceptible to RF heat than pupal and adult stages exposed to the radio frequency at 420 watts for 60 seconds. The rank of RF tolerance is: adult>pupal>larval= egg stages. Other stored product insects such *as* adult of *S. oryzae, S. zeamais* and *Rhyzopertha dominica* have demonstrated tolerance to RF heat (Faikrajaipuen et al. 2011; Sumetha et al., 2009; Wangsapa et al., 2015). On the other hand, eggs of Angoumois grain moth, *Sitotroga cerealella* (Buapud et al., 2012) and the cowpea weevil, *Callosobruchus maculatus* (Na Pijit et al., 2011*)* which both lay eggs on rice kernels and mung beans are RF heat tolerance and is likely due to their adherence to the seeds.

The treatments; 60°C for 3 minutes, and 65°C for 2 and 3 minutes caused 100% mortality of larvae. These results can be associated with greater water content in larvae which would cause higher temperature to kill insects due to high oscillation of water molecules or polar molecules as presented by Wangsapa (2016) who reported that moisture contents of *S. oryzae* larvae (66.53±0.8% mc) was greater than pupae(64.21±0.9%) and adults (46.96±0.4% mc), respectively.

Adult of cigarette beetle showed 51% mortality when exposed to RF at 55°C for 1 minute while the pupal stage showed 71.19%, but both adult and pupal stage were completely killed when exposed to treatments of 65°C for 3 minutes. This experiment would confirm that pupal and adult stages of cigarette beetle are the most tolerant to RF heat treatment. From previous experiment done by Nutapong (2012) the result also showed adult and pupal stages were the most tolerant to heat greater than egg and larval stages compared to the heat of 104°C for 180 seconds. Since the treatment of 65°C for 3 minutes caused 100% mortality of tolerant stage it can be recommended for commercial scale treatment. Thus, this result was able to minimize the heat of RF on controlling of all stages of cigarette beetle with 100% of the insect control.

While no work on the effects on progeny were performed in this work, Wangsapa (2016) found that thirty rice weevil, *S. oryzae* treated with 27.12 MHz in laboratory condition were able to produce 13.25±4.5 insects compared with the untreated control insects that produced 26.75±0.8 offspring. Srikam et al. (2014) also determined the effect of RF treatment on sawtoothed grain beetle, *O. surinamensis* infested rice. Their results found that when insects were treated with RF, the number of progeny produced from survivors was significantly less than in untreated controls. If adult of *O. surinamensis* were completely killed by RF heat treatment, there was no progeny production found.

This indicates that even if insects have the opportunity to lay eggs before the heat treatment was completed, all adults and their eggs would die when exposed to heat.

In dried tobacco process, there is the lamina re-drier process of tobacco which consisted of 4 continuous drier chambers at 80-88°C for 3 min in each chamber (personal contact), so this treatment temperature would have less effect ton tobacco quality. The data was supported by research done in Nutapong (2012) which treated dried tobacco with 27.12 MHz of RF energy at 104°C for 3 min caused slightly changed for Burley green leaf tobacco (B2F grade). In dried tobacco treated with RF the nicotine content was 4.13% significantly higher than in untreated control (3.18%). The chemical and physical properties of RF-treated tobacco were remained in Burley green leaf tobacco standard. According this experiment, the death profile of cigarette beetle with 65°C for 3 minutes has potential to apply on dried tobacco with less effect to tobacco quality.

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