【Original Articles】

# Comparison of roasted coffee beans and black tea leaves for oviposition stimuli in cigarette beetle

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**Abstract**

Oviposition stimulation in the cigarette beetle using roasted coffee beans and black tea leaves was compared. Roasted coffee beans and black tea leaves were sequentially extracted by hexane, chloroform, butanol, methanol, and 20% methanol in water. Methanol and 20% methanol in water extracts of black tea leaves and chloroform, butanol, methanol, and 20% methanol in water extracts of roasted coffee beans exhibited oviposition stimulant activity. The activity was not present in the chloroform extract of black tea leaves, and we could not detect catechol, which had been identified as an active component of the chloroform extract of roasted coffee beans. Methanol extracts of both roasted coffee beans and black tea leaves, which revealed high activity, were further separated by reverse-phase column chromatography. Single fractions from the coffee extract did not show any activity; on the other hand, two fractions from the black tea extract still showed significant activity.

**Key words:** *Lasioderma serricorne*, roasted coffee bean, black tea leaf, oviposition stimulant

## INTRODUCTION

The cigarette beetle *Lasioderma serricorne* (Fabricius) is a serious global pest that feeds on stored foods. The beetle larvae damage a wide range of dried plant and animal materials (Ashworth, 1993; Hill, 2002). In contrast, adult feeding is limited (Ashworth, 1993). Adults live for 2–6 weeks, and adult females lay approximately 100 eggs (Hill, 2002). The adult beetles oviposit onto dried food material leading to damage caused by feeding activity of the resulting larvae (Ashworth, 1993). Under laboratory conditions, they oviposit onto materials like roasted coffee beans and tea leaves, on which larvae cannot grow (Hori et al., 2011). Therefore, it may be possible to reduce the pest population by inducing gravid females to lay their eggs on substrates unsuitable for larval development (Nagasawa et al., 2014). Our previous chemical analyses of coffee and tea identified catechol as an active component from the chloroform extract of roasted coffee beans (Nagasawa et al., 2014). In this study, first we tried to detect catechol in the chloroform extract of black tea leaves, then investigated the methanol extracts of roasted coffee beans and black tea leaves, in preparation for further isolation of active components with higher polarity.

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## MATERIALS AND METHODS

### 1. Insects

The cigarette beetles were obtained from cultures at the Leaf Tobacco Research Center of Japan Tobacco Inc. (Tochigi, Japan) in 2007 and maintained in our laboratory. They were reared on corn flour (Nippon Flour Mills Co., Tokyo, Japan), containing 10% dry brewer's yeast (Ebios®; Asahi Food & Healthcare Co., Tokyo, Japan), and maintained at 25 ± 1℃ under a photoperiod of 16:8 L:D.

### 2. Extraction and fractionation of roasted coffee beans and black tea leaves

The roasted coffee bean powder (497.30 g) and the black tea leaves (500 g) were separately soaked in hexane (2 L) for 24 h and then filtered. Extractions were conducted 10 times. The residue was then soaked in chloroform ($CHCl_3$) 10 times in the same manner as that for hexane. Extractions were subsequently conducted using 1-butanol (BuOH), methanol (MeOH), and 20% MeOH in water in the same manner as for $CHCl_3$. Each extract was evaporated to dryness under reduced

pressure at <40℃.

The $CHCl_3$ extract of black tea leaves was fractionated by normal-phase silica-gel column chromatography (Wako-gel C-200, Wako Pure Chemical Industries, Osaka, Japan, 200 g, 46 mm inner diameter column). Fractions were sequentially eluted with 10%, 20%, and 100% ethyl acetate in hexane and finally with MeOH (1500 ml each). Each fraction (Fr.) was evaporated to dryness at reduced pressure at <40℃.

The MeOH extracts of roasted coffee beans and black tea leaves were respectively fractionated by reverse-phase [octadecyl silica-gel (ODS)] column chromatography (Wako-gel 100C18, Wako Pure Chemical Industries, Osaka, Japan, 200 g, 30 mm inner diameter column). Fractions were sequentially eluted with 0%, 25%, 50%, 75%, and 100% MeOH in water (500 ml each). For the black tea leaf extraction, two additional liters of MeOH were reverse-flowed through the column. Each fraction (Fr.) was evaporated to dryness under reduced pressure at <40℃. All processes for extraction and fractionation are shown in **Fig. 1**.

### 3. Analysis of catechol by Gas Chromatography-Mass Spectrometry

The absence of catechol in the black tea leaf extract was confirmed by single ion monitoring (SIM, *m/z* 64 and 110) of gas chromatography/mass spectrometry (GC/MS) analysis. The fraction with the polarity corresponding to catechol (20% ethyl acetate in hexane of the $CHCl_3$ extract)

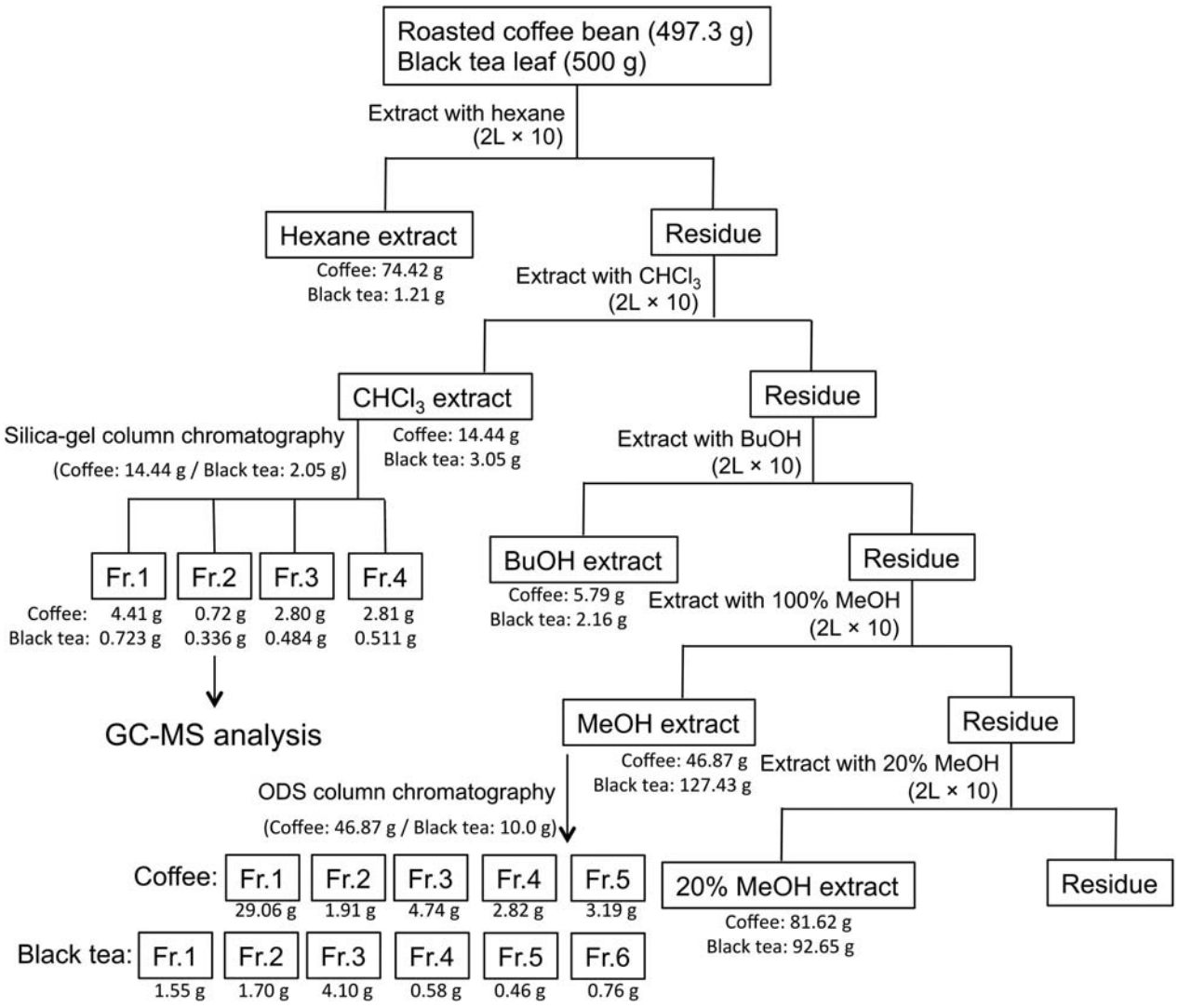


**Fig. 1 Extraction and fractionation for roasted coffee beans and black tea leaves.**

and authentic catechol (Tokyo Chemical Industry Co. Ltd, Tokyo, Japan) were analyzed by a Shimadzu GCMS-QP2010 Ultra, equipped with a DB-5MS column (30 m × 0.25 mm i.d., 0.25 μm film thickness, J&W, Santa Clara, CA). The temperature of the column oven was programmed to increase by 3℃/min from 60℃ to 200℃, by 10℃/min from 200℃ to 300℃, and then held for 10 min. The injector, detector, and interface temperatures were 220℃, 200℃, and 240℃, respectively.

### 4. Oviposition bioassay

Each extract and fraction of the extract from the roasted coffee beans or the black tea leaves was dissolved in the same solvent as used for extraction. The concentrations of the solutions were 1 g coffee-/tea-equivalent/ml. One milliliter of each solution was applied to two sheets of filter paper (55 mm diam.). Control filter paper was prepared similarly, using the solvent alone. After air-drying, the two sheets of filter paper were placed on the bottom of a glass Petri dish (70 mm diam., 20 mm height). After confirmation of copulation, a pair (male and female) of beetles was released into the Petri dish within 24 h of emergence from the corn flour feed. The dishes were kept under rearing conditions for 6 or 7 days, and then the number of eggs laid was counted. Eight replicates (eight separate Petri dishes) were used for each treatment.

### 5. Statistical analyses

We performed statistical analyses using R v. 3.2.1 for Mac OS X (R Core Team, 2015). The mean number of eggs was compared using Welch's *t*-test and Dunnett's test, for the extracts and the fractions of chromatography, respectively, after log transformation [log ($x$ + 0.5)].

## RESULTS

### 1. Oviposition response to extracts of black tea leaves

The number of eggs laid on the MeOH and 20% MeOH extract was greater than the number laid on the control ($P < 0.01$) (**Fig. 2**). The number of eggs laid on other extracts was not significantly different from the number laid on the control ($P > 0.05$) (**Fig. 2**).

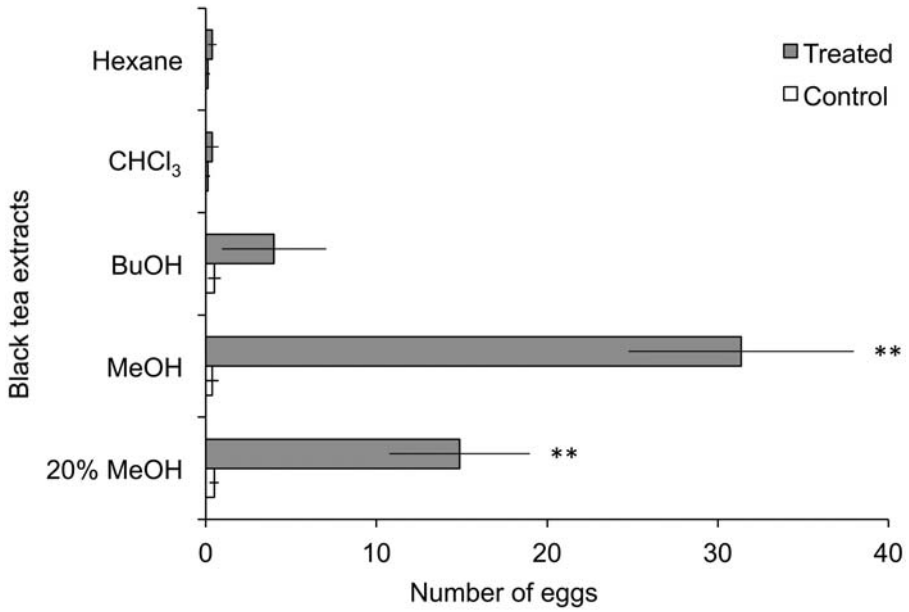


**Fig. 2 Number of eggs laid by cigarette beetles on extracts using various organic solvents. Each extract was provided on filter paper to a pair (male and female) of beetles for 6 days. The bars with the double asterisks are different from the control [Welch's *t*-test after a log (x + 0.5) transformation, $P < 0.01$].**

**2. Oviposition response to fractions of MeOH extract**

The female beetles did not lay significantly larger number of eggs on any single fractions of the MeOH extract of roasted coffee beans than the solvent control ($P > 0.05$), although they laid more eggs on the mixture of all fractions ($P < 0.05$) (**Fig. 3**). The MeOH extract of black tea leaves, on the other hand, elicited significant oviposition stimulant activity after fractionation. The number of eggs laid on two fractions Fr. 1 and Fr. 5, in addition to the mixture of all fractions, was significantly greater than that laid on the control ($P < 0.05$) (**Fig. 4**).

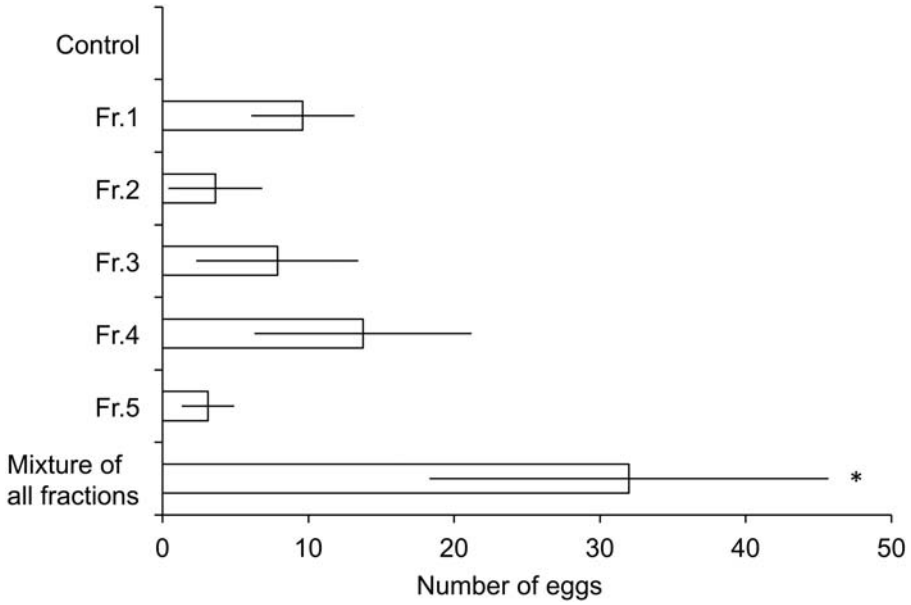


**Fig. 3 Number of eggs laid by cigarette beetles on fractions of a MeOH extract of roasted coffee beans. Each fraction was provided on filter paper to a pair (male and female) of beetles for 6 days. The bar with the asterisk is different from the control [Dunnett's test after a log (x + 0.5) transformation, $P < 0.05$].**

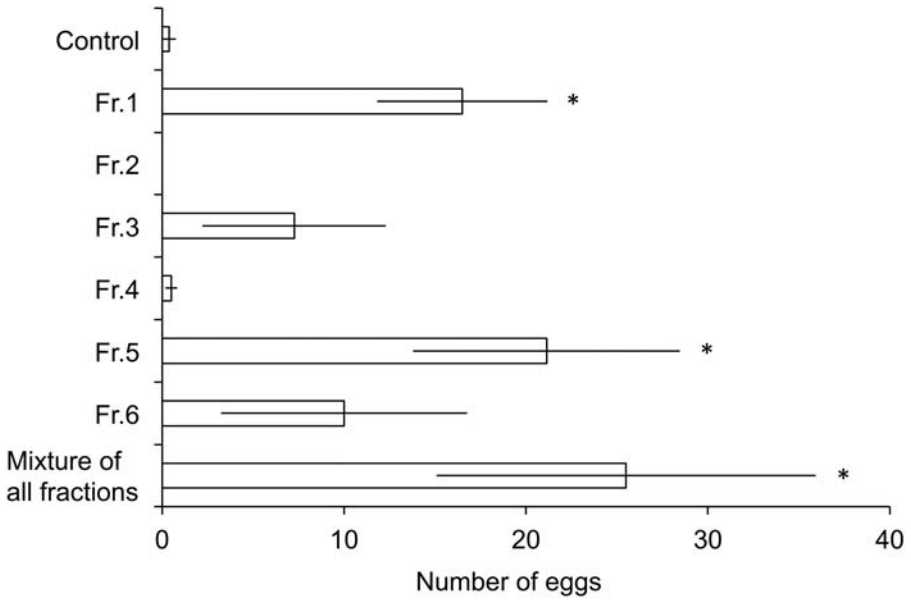


**Fig. 4 Number of eggs laid by cigarette beetles on fractions of a MeOH extract of black tea leaves. Each fraction was provided on filter paper to a pair (male and female) of beetles for 7 days. The bars with the asterisk are different from the control [Dunnett's test after a log (x + 0.5) transformation, $P < 0.05$].**

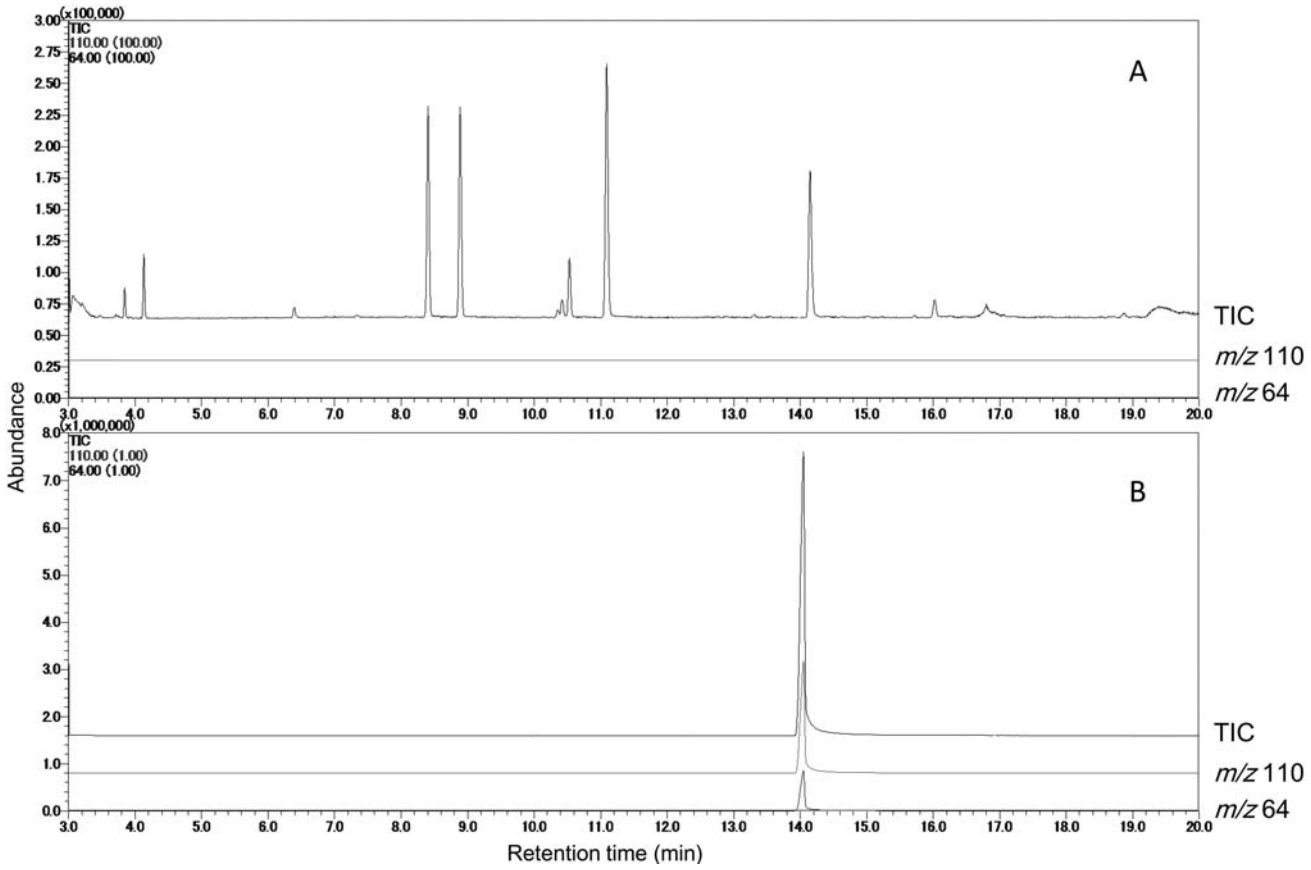


**Fig. 5 Total ion current chromatogram and selected-ion monitoring chromatogram (*m/z* 64 and 110). A: Fraction 2 of a $CHCl_3$ extract of black tea leaves. B: Authentic sample of catechol.**

### 3. Analysis of catechol in the extract of black tea leaf

In the fraction with the polarity corresponding to catechol, ions specific to catechol (*m/z* 64 and 110) were not detected around the corresponding retention time on the GC (**Fig. 5**).

## DISCUSSION

In the previous study, the authors found oviposition stimulant activity on the $CHCl_3$, BuOH, MeOH, and 20% MeOH extracts of roasted coffee beans and determined catechol as an active component of the $CHCl_3$ extract (Nagasawa et al., 2014). In this study, we also found oviposition stimulant activity on the MeOH and 20% MeOH extracts of black tea leaves, but could not find any activity on the $CHCl_3$ and BuOH extracts (**Fig. 2**). We further confirmed that black tea leaves did not contain detectable amount of catechol (**Fig. 5**). Catechol is produced by the decomposition of *O*-caffeoylquinic acids (Clifford, 1979; Haffenden and Yaylayan, 2005; Lang et al., 2006; Müller et al., 2006). Therefore, the cigarette beetle, which is a pest in after-harvest stored product, may use this degradation compounds as an oviposition stimulant. Thus, we expected that the catechol is a common oviposition stimulant in various foods. However, catechol is only one of the oviposition stimulants for the beetle and various compounds may affect oviposition of the beetle. Oviposition stimulant activity on the MeOH extracts of both roasted coffee beans and black tea leaves declined after fractionation. Therefore, multiple highly polar compounds would have stimulant activity. Difference in yields of extracts indicates that difference of amount of active compounds may be responsible for difference of activity on the fractions of the MeOH extracts between coffee and black tea.

This study indicates that a wide range of

oviposition targets is likely caused by a wide range of active compounds, rather than a common compound contained in the oviposition targets. This may be one of the factors affecting the wide range of feeding damage caused by the beetle. We need to study oviposition stimulants in the roasted coffee beans and the black tea leaves further. If we can identify these compounds and determine effective combination of active compounds, we may be able to develop a stable oviposition stimulant for the pest. This is expected to regulate the cigarette beetle populations by inducing the beetle to lay most of their eggs on substrates unsuitable for larval development.

**ACKNOWLEDGEMENTS**

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【原著】

# 焙煎コーヒー豆と乾燥紅茶葉におけるタバコシバンムシに対する産卵刺激因子の比較

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**摘 要**

焙煎コーヒー豆と紅茶葉に含まれるタバコシバンムシに対する産卵刺激因子の比較を行った．焙煎コーヒー豆からはカテコールが産卵刺激物質として明らかになっているが，同様の方法で紅茶葉を抽出したところ，メタノール，20%メタノール抽出物に産卵刺激活性が認められた．コーヒーにおいてカテコールを分離したクロロホルム抽出物画分と同様の方法で，活性が認められなかった紅茶葉のクロロホルム抽出物から分離した画分を GC-MS 分析したところ，カテコールは検出されなかった．コーヒーおよび紅茶のいずれにおいても活性が認められたメタノール抽出物を分画すると，コーヒーではすべての画分を混合しないと活性が現れないが，紅茶では単独で活性を示す画分があった．

**Key words:** タバコシバンムシ, 焙煎コーヒー豆, 紅茶葉, 産卵刺激物質

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