



## The effectiveness of vegetable oil formulations in reducing oviposition of *Bactrocera dorsalis* Hendel (Diptera: Tephritidae) in large red chili fruits

Efektivitas formulasi minyak nabati dalam menghambat oviposisi *Bactrocera dorsalis* (Diptera: Tephritidae) pada tanaman cabai

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### ABSTRACT

Chili peppers (*Capsicum annuum*), in their many varieties, constitute a culturally and economically important horticultural crop in a number of countries. The Indonesian cayenne large red chili (*Capsicum annuum* var. *annuum*) is used widely in Indonesia mainly in cooking. There have been reports of increased infestation of large red chili by insect pests, particularly fruit flies. The aim of this study was to investigate the effectiveness of five edible vegetable oils (palm oil, coconut oil, soybean oil, corn oil, and candlenut oil) and one non-edible vegetable oil (neem oil) in reducing landings, oviposition, and infestation by the oriental fruit fly (*Bactrocera dorsalis* Hendel) in large red chili fruits. This lab-based experiment entailed exposure of large red chili fruits to 20 mature *B. dorsalis* females (14–21 days old) inside a 15-l plastic container. Six separate containers each held 10 large red chili fruits with a single oil treatment in each. Prior to exposure, each of the treated and control large red chili fruits was punctured once with a needle in order to create an opening for oviposition. Results indicate that the coconut oil formulation was most effective in preventing damage from *B. dorsalis* females, and reducing fruit fly landings, oviposition, and infestation.

**Key words:** coconut oil, edible oil, irritant, neem oil, oriental fruit fly

### ABSTRAK

Cabai (*Capsicum annuum*) dan berbagai varietasnya merupakan tanaman hortikultura yang memiliki nilai penting bagi budaya dan ekonomi di berbagai negara. Cabai merah besar Indonesia (*Capsicum annuum* var. *Annuum*) digunakan secara luas di Indonesia terutama untuk masakan. Peningkatan infestasi serangga hama pada cabai merah besar telah dilaporkan, terutama lalat buah. Tujuan dari penelitian ini adalah untuk mengetahui keefektifan lima minyak nabati yang dapat dikonsumsi/dimakan (minyak sawit, minyak kelapa, minyak kedelai, minyak jagung, dan minyak kemiri) dan satu minyak nabati yang tidak bisa dikonsumsi/dimakan (minyak nimba) dalam mengurangi kunjungan, oviposisi, dan infestasi oleh lalat buah oriental (*Bactrocera dorsalis* Hendel) pada buah cabai merah besar. Percobaan berbasis laboratorium ini menggunakan paparan buah cabai merah besar untuk 20 individu *B. dorsalis* betina dewasa (14–21 hari) di dalam wadah plastik 15-l. Enam wadah terpisah masing-masing berisi 10 buah cabai merah besar dengan satu perlakuan minyak di masing-masingnya. Sebelum terpapar, buah cabai merah besar ditusuk sekali dengan jarum pada masing-masing perlakuan dan kontrol untuk menciptakan pembukaan untuk oviposisi. Hasil menunjukkan bahwa formulasi minyak kelapa paling efektif dalam mencegah kerusakan dari *B. dorsalis* betina, dan mengurangi kunjungan lalat buah, oviposisi, dan infestasi.

**Kata kunci:** iritasi, lalat buah oriental, minyak kelapa, minyak nabati, minyak neem

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## INTRODUCTION

Chili peppers (*Capsicum annuum*), in their many varieties, constitute a culturally and economically important horticultural crop in a number of countries; however, large red chili (*Capsicum annuum* var. *annuum*) production is often hampered by insect pests, as well as plant pathogens. Of these pests, the fruit fly is considered to be the most destructive to large red chilis. Fruit fly eggs are laid inside large red chili fruits, growing into larvae that feed on the fruit flesh, causing them to rot and/or drop to the ground (Wingsanoi & Siri 2012). The fruit fly species which most commonly attack large red chili fruits include the oriental fruit fly (*Bactrocera dorsalis* Hendel) (Liquido et al. 2015) and the solanum fruit fly (*Bactrocera latifrons* Hendel) (Wingsanoi & Siri 2012).

To reduce fruit fly infestation on large red chili fruits, farmers often use synthetic insecticides. Dimethoate and fenthion are two synthetic insecticides known to be effective against both the larvae and adult fruit fly (Dominiak & Ekman 2013; Rahman & Broughton 2016). However, there are concerns about the safety of these two insecticides for human consumption and exposure. Budreau & Singh (1973) reported the effect of dimethoate and fenthion on reproduction in the mouse. Therefore, effective but less toxic alternatives are needed for controlling fruit fly attacks on large red chili fruits.

Pesticides of plant origin may be effective alternatives to synthetic insecticides to guard against fruit flies. Various plant products including plant extracts (Silva et al. 2012), essential oils (Benelli et al. 2012) and vegetable oils (Nunes et al. 2015) have been shown to have bioactive properties that defend against fruit fly damage. Vegetable oils are easier to produce than plant extracts and essential oils. Vegetable oils can be separated from plant materials through mechanical pressing (Uquiche et al. 2008) whereas the extracts are separated through organic solvent extraction (Pascual-Villalobos & Robledo 1998) and essential oils through water/steam distillation (Boutekedjiret et al. 2003). Hence, the cost of producing vegetable oils is often cheaper than other plant products. Of course, the price of a plant product also depends on other factors such as

the cost to produce the plant material (leaves, oil seeds, etc.) and the amount of oil or other extract contained in said plant material.

There are two types of vegetable oils, i.e. non-edible and edible oils. Neem oil is an example of a non-edible vegetable oil that has been reported to prevent the damaging activities of fruit flies (mainly oviposition) (Khattak et al. 2009; Sharma & Kumar 2016). The widespread use of neem products for insect control purposes, however, is often limited by the high cost of neem seeds (Isman 2004).

Edible oils may also be effective for insect control, and are less expensive and more readily available. However, little is known about the characteristics of edible vegetable oils and how they might protect against fruit fly infestation. A previous laboratory study showed that the use of safflower oil and palm oil were able to reduce oviposition by queensland fruit flies (*Bactrocera tryoni* (Froggatt)) on apples, although this was true only during an experimental choice test (Hidayat et al. 2013). We do not know if this will be true in field conditions. The aim of the present study is to investigate the effectiveness of five edible vegetable oils (palm oil, coconut oil, soybean oil, corn oil, and candlenut oil) and a non-edible vegetable oil (neem oil) in reducing *B. dorsalis* landing, oviposition, and infestation in large red chili fruits. These five edible vegetable oils were chosen because of their availability and cost.

## MATERIALS AND METHODS

### Vegetable oil formulations

Six vegetable oil formulations were developed and investigated for anti-pest properties in this study. Because we expect farmers will only have access to commercially available products, some oils were procured as “off the shelf” consumer products rather than freshly derived from plant materials. These included palm oil (Filma<sup>®</sup>), coconut oil (Barco<sup>®</sup>), soybean oil (Happy Soya Oil<sup>®</sup>) and corn oil (CCO<sup>®</sup>), which were purchased from a local supermarket. All the oils have not passed their expiration dates. Candlenut oil and neem oil were separated mechanically using a spindle press from oil seeds harvested from the trees grown

in Jatinangor (West Java Province, Indonesia) and Situbondo (East Java Province, Indonesia), respectively. Each vegetable oil was mixed with surfactants (polysorbate 80, sorbitan monooleate) and oleic acid; 4 : 1 : 5; w/w) with the resultant vegetable oil content in each formulation 70% (w/v).

### Oriental fruit fly

Oriental fruit fly (*B. dorsalis*) individuals were obtained as pupae from a colony kept at the Pest Forecasting Institute, Jatisari, Indonesia. This fruit fly colony was previously classified as *Bactrocera papayae*. However, a recent review suggests that there is insufficient evidence to maintain *B. papayae* as a separate biological species from *B. dorsalis*, its senior synonym (Schutze et al. 2015). Therefore insects from this colony were re-named *B. dorsalis*, and deemed appropriate for use in our study of *B. dorsalis* infestation prevention.

The colony-sourced fruit fly pupae were raised to adulthood in the lab. For this experiment, the resulting fruit fly adults were kept in aluminum-framed, mesh-covered cages (50 cm x 50 cm x 50 cm) and maintained at room temperatures (25–28 °C), 70–85% RH, with a 12 : 12 (light : dark) photoperiod. Adults were provided with sugar cubes ( $\pm 20$  g), yeast hydrolysate (Affymetrix Inc., USA) paste ( $\pm 10$  g), and also water ( $\pm 200$  ml). 2<sup>nd</sup> generation eggs were harvested using an oviposition device comprised of a clear plastic container ( $\pm 200$  ml) filled with 20 ml of mango-flavored fruit drink (Nutrisari<sup>®</sup>) and covered with a layer of plastic film held on with a rubber band. Adult female fruit flies laid their eggs through the plastic film (perforated), and these eggs were then collected, washed with water, and placed on filter paper. Next, the egg-filled filter paper was transferred to a plastic tray containing carrot-based larval feed. The larval feed blend was formulated as follows: fresh raw carrots (600 g), mauri-pan<sup>®</sup> yeast (30 g), nipagin (3 g), propionic acid (4 ml), and water (500 ml), combined and pureed using a food processor. The hatched larvae were raised in the plastic tray until late third instar, when the tray –including the larval feed– was stored inside a plastic container with a bed of sand for pupation. Once the fruit flies reached pupal stage, they were harvested by sieving the sand bed. The collected pupae were raised to adulthood inside a

1-l plastic container with moistened sand. Next, the resulting adults were transferred to aluminum-framed, mesh-covered cages (50 cm x 50 cm x 50 cm), approximately 400–500 adults per cage, and supplied with yeast hydrolysate, sugar cubes, and water as food. Yeast hydrolysate was mixed with water to form a paste, and placed on cardboard in the cage. Sugar cubes were placed inside the cage in a clear, flexible plastic beverage lid (9 cm diameter) lined with tissue paper. Water was provided by placing folded tissue paper soaked with bottled spring water in a plastic cup. These adults were kept in rearing cages for 13–20 days prior to experimentation and fresh food and water was provided twice a week. At one day prior to experimentation, females of *B. dorsalis* were transferred from rearing cages to experimental cages made from 15-l clear plastic containers covered with cloth mesh for ventilation. Yeast hydrolysate paste (on a sheet of semi-rigid plastic) and a sugar cube were placed on top of the experimental cage so that the fruit flies could feed on the food from inside of the cage. Water was sprayed onto the inside wall of the plastic containers.

### Application of vegetable oil formulations on large red chili fruits to reduce fruit fly oviposition

Six vegetable oil formulations at a concentration of 10 ml l<sup>-1</sup> were tested for their effects on the oviposition of *B. dorsalis* in large red chili fruits. Each formulation was diluted with water in a glass beaker to form an emulsion. Large red chili fruits were harvested from the field while still green (unripe) and examined for any signs of fruit fly infestation. For each treatment, ten of these unripe, large red chili fruits (confirmed to be fruit-fly-free) were dipped for 10 seconds in one vegetable oil emulsion and air dried for 30 minutes. This was repeated for each vegetable oil type, for a total of 6 treatment groups of 10 fruits each, treated with a different vegetable oil. As a control, 10 large red chili fruits were dipped in water and then air dried. Prior to exposure to fruit fly females, all treated and control large red chili fruits were punctured with an entomological needle (1 puncture/fruit). One obvious mechanism by which oil might prevent oviposition is by preventing females from making oviposition punctures. Puncturing the fruit

in advance of exposure to fruit flies allowed us to ascertain whether the vegetable oil formulations may prevent oviposition in some other way. Once prepared (dipped and punctured), 10 treated large red chili fruits were placed inside an experimental cage, containing 20 mature *B. dorsalis* females (14–21 days old). The experiment was arranged in a complete block design with four replications.

Researchers observed the number of fruit fly landings, punctures, eggs laid, and fruits infested. A fruit was categorized as infested if at least one fruit fly egg was found inside the fruit. Immediately after introduction of the chili fruit to the fruit fly cages, fruit fly landings on large red chili fruits were counted during observation periods of one minute, at 30 minute intervals for six hours, for a total of 12 observation periods. Fruit flies that spent only a short period of time alighted on the fruits (< 5 seconds) and quickly moved away were not counted as landing. The other parameters were measured later, 24 hours after large red chili fruit exposure to *B. dorsalis*, a time frame generally considered sufficient for oviposition to occur (Hidayat et al. 2013). The presence of fruit fly punctures was detected through careful visual examination of the skins of exposed large red chili fruits. Each oviposition puncture made by the exposed fruit flies was counted (excluding the pre-punctures made by the researchers). Each exposed chili fruit was dissected in order to count the number of eggs laid within. From this data, an oviposition deterrence index (ODI) was calculated using the formula  $[(C - T) / (C)] \times 100\%$ , where C and T are the number of fruit fly eggs counted in control and treated groups, respectively. Fruit fly infestation was calculated using the formula  $[(A) / (A + B)] \times 100\%$ , where A and B were the number of large red chili fruits infested and not infested by fruit flies, respectively.

### Statistical analyses

Data were first checked for normality and homogeneity of variance. When data were not normally distributed and variances were not homogenous, a data transformation was applied, prior to subjecting data to analyses with GLM ANOVA. Identification of significant difference for the treatments was conducted using Tukey's test. GLM ANOVA and its post-hoc test were

carried out using a statistical software package, *Minitab* version 16. If the required assumptions for ANOVA were not satisfied, data were analyzed using the non-parametric Friedman test and Nemenyi post-hoc test. The non-parametric tests were carried out in *R* statistical software, version 3.3.1 using the Pairwise Multiple Comparison of Mean Ranks (PMCMR) package.

## RESULTS

### Fruit fly landings

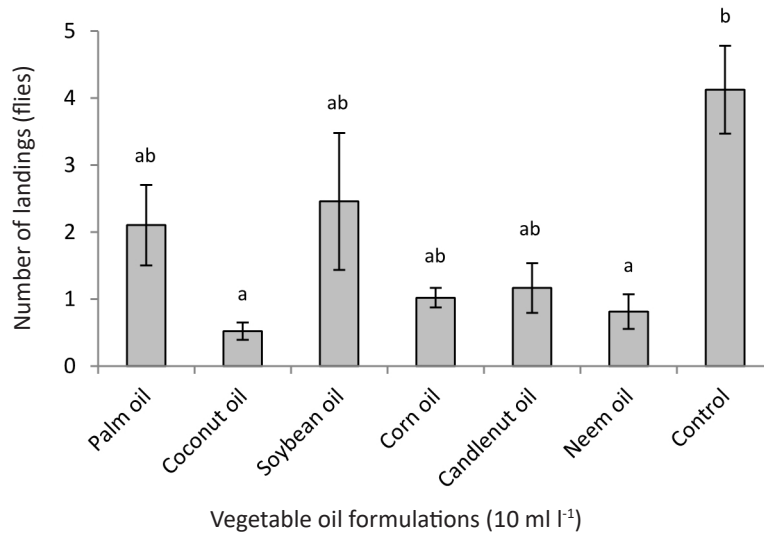
The results of this study showed significant effects for coconut oil and neem oil treatments on the average number of fruit fly landings on the fruits ( $F_{6,18} = 4.19$ ;  $P = 0.008$ ) (Figure 1). The number of landings on fruit in these two treatment groups averaged just one compared to 4 landings in the control group. No significant difference in landings was observed for large red chili fruits that were treated with the other vegetable oil formulations, compared to the control group.

### Fruit fly punctures and laid eggs

There was no significant difference in the number of fruit fly punctures (oviposition sites) found on large red chili fruit in any of the treatment groups compared to the control ( $\chi^2 = 7.51$ ,  $df = 6$ ,  $P = 0.276$ ) (Table 1). However, results for three of the treatments (coconut oil, corn oil, and neem oil) showed significantly fewer eggs laid in treated fruits ( $F_{6,18} = 4.83$ ,  $P = 0.004$ ) than in control fruit. Among these, the coconut oil formulation was found to be the most effective, with 175.5 eggs laid in treated fruits compared to 767.3 eggs in the control group (translating to an Oviposition Deterrence Index of 76.1%).

### Fruit fly infestation

The coconut oil treatment group also experienced significantly lower infestation rates by fruit flies ( $\chi^2 = 13.43$ ,  $df = 6$ ,  $P = 0.037$ ) compared to the control (Figure 2). The group treated with the coconut oil formulation experienced a 50% infestation whereas the control group infestation rate was 100%. Test groups of chili fruit treated with other vegetable oil formulations did not show any significant difference in fruit fly infestation.



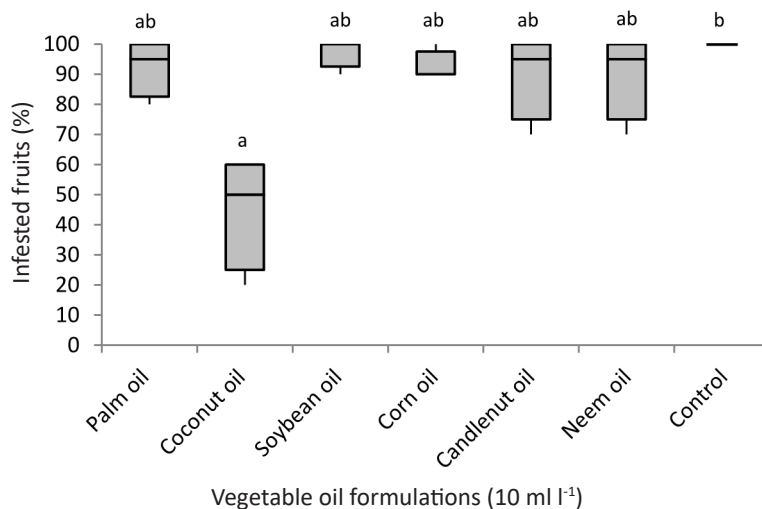
**Figure 1.** Fruit fly landings on treated and untreated large red chili fruits (Means  $\pm$  SEM). Data were Box-Cox ( $X + 0.83$ ) transformed ( $\lambda = -0.50$ ) prior to GLM ANOVA analysis. Bars with a different letter indicate significant differences according to Tukey’s test ( $\alpha = 0.05$ ).

**Table 1.** Number of fruit fly punctures and eggs laid in treated vs. untreated large red chili fruits

Vegetable oil formulations (10 ml l <sup>-1</sup> )	Fruit fly punctures (Median, Q1–Q3)*	Eggs laid (Means $\pm$ SEM)	Oviposition Deterrence Index (%)**
Palm oil	0.5 (0.0–1.0)	561.2 $\pm$ 125 ab	28.1 $\pm$ 9.8
Coconut oil	1.0 (0.0–2.0)	175.5 $\pm$ 12.4 a	76.1 $\pm$ 3.1
Soybean oil	1.5 (1.0–2.0)	496.0 $\pm$ 145 ab	36.1 $\pm$ 21.1
Corn oil	1.5 (0.25–2.0)	370.0 $\pm$ 106 a	52.8 $\pm$ 10.3
Candlenut oil	0.5 (0.0–1.75)	460.8 $\pm$ 102 ab	39.7 $\pm$ 11.4
Neem oil	0.5 (0.0–1.75)	367.0 $\pm$ 80.7 a	54.0 $\pm$ 5.0
Control	2.5 (1.25–3.0)	767.3 $\pm$ 96.9 b	-

\*No significant differences according to the non-parametric Friedman’s test ( $\alpha = 0.05$ ). Means in the same column followed by a different letter are significantly different according to Tukey’s test ( $\alpha = 0.05$ ).

\*\*Calculated using the formula  $[(C - T)/(C)] \times 100\%$ . -: not applicable.



**Figure 2.** Box plots of fruit fly infestation on large red chili fruits. Box plots show first quartile (Q1), median, and third quartile (Q3). The whiskers extend down to the minimum value and up to the maximum value. Box plots with a different letter indicate significant differences according to Friedman’s test followed by a Nemenyi post-hoc test ( $\alpha = 0.05$ ).



## DISCUSSION

Results of this laboratory study indicate that the application of a coconut oil treatment to large red chili fruits led to fewer fruit fly landings, eggs laid, and infested fruits. Our findings suggest that this particular edible vegetable oil may be effective for control of fruit fly infestation, and may be a viable alternative to non-edible neem oil. This is important because locally available food oils are less expensive than Neem oil and can more easily and cost-effectively provide protection from fruit-fly infestation in Indonesia. This finding is in accordance with previous research showing protective effects of edible oil against fruit infestation. One previous laboratory study testing the effectiveness of edible oils against fruit-fly infestation showed that the number of queensland fruit fly *B. tryoni* eggs laid in apples treated with either safflower or palm oil at a concentration of 10 ml l<sup>-1</sup> was 56.4% and 42.5% lower than in the control group, respectively, though only in a choice test (Hidayat et al. 2013). Rapeseed oil is another edible vegetable oil that has been reported to be active against fruit fly oviposition. The application of a rapeseed oil product at a concentration of 1% significantly reduced oviposition by the European cherry fruit fly *Rhagoletis cerasi* (Linnaeus), in cherry fruits for up to 3 days, but the effect disappeared within 3 to 6 days after application (Daniel 2014). The current research did not test the duration of protective effects for oils used against fruit fly infestation. This should be a focus of future research.

Previous studies suggested that the application of a vegetable oil can create a physical barrier (slippery effect) on the treated fruits and thus prevent the fruit fly from making oviposition punctures (Hidayat et al. 2013; Daniel 2014; Hidayat et al. 2014). In this study, however, all treated and untreated large red chili fruits were prepunctured with an entomological needle prior to exposure to the fruit flies. Therefore, the significantly lower number of eggs laid in the coconut-oil-treated chili fruits compared to the control group could not be attributed merely to the prevention of fruit flies from making oviposition punctures.

Another potential mechanism for reduced infestation in treated fruits could be the repellent effect of chemical compounds in the treatment

oils. Dethier et al. (1960) defined a repellent as a chemical that causes insects to make oriented movements away from its source. Arguing against a strong repellent effect is the fact that vegetable oils, including coconut oil, are mainly composed of non-volatile substances rather than the volatile compounds that might typically attract or repel insects. The main component of crude vegetable oil is triacylglycerol (about 95%) and the minor components are free acids, monoacylglycerols, diacylglycerols, phospholipids, free and/or acylated sterols, tocopherols, and hydrocarbons such as alkanes, squalene, and carotenes (Gunstone 2006). Crude coconut oil is usually refined physically or chemically to remove undesirable minor components including volatile components (Amri 2011), rendering refined commercial coconut oil even more benign. A study by Dayrit et al. (2007) showed that commercial samples of refined, bleached and deodorized coconut oil (RBD CNO) had an average volatile matter of only 0.03%, whereas virgin coconut oil (VCO) had an average volatile matter of 0.13% (w/w, measured at 120 °C). Regardless, the emission of volatiles from coconut oil occurs only when the temperature of coconut oil is above its smoke point (175 °C) (Katragadda et al. 2010).

Another possible explanation for the lower rates of successful oviposition on coconut oil-treated chili peppers, is the creation of a film on the fruit surface which traps or reduces the release of volatile chemicals that attract fruit fly females. This is separate from the physical barrier effect that an oily film might confer (an effect rendered moot by the pre-puncturing of our experimental fruits). Coconut oil is known to be an effective coating for fruit to prevent release of, or exposure to, volatile compounds that trigger ripening. It is used commonly for precisely this purpose to block volatile chemicals and extend the shelf life and quality of produce (Pandey & Joshua 2010). Indeed, fruit are often coated with edible materials such as lipids, waxes and resins to create a barrier to transmission of gases including fruit aromas (Aguirre-Joya et al. 2016). While it is possible that coconut oil treatment reduced emission of volatile chemicals that would attract female fruit flies, in our study, each treated chili pepper was pre-punctured prior to exposure to female fruit flies, which would provide a means for volatiles to escape. There were 10 punctured large red

chili fruits provided to 20 fruit fly females in each experimental cage, and these punctures are likely to emit chili fruit volatiles that could be detected by the fruit flies. Therefore, the reduction in the number of fruit fly visits, laid eggs and infested fruits in the current study cannot be attributed solely to the coating effect of coconut oil and blocking of chili fruit volatiles, as they would be only partially blocked if at all.

The results of the current study suggest it may be possible to use coconut oil to control *B. dorsalis* infestation in large red chili fruits. We found a 50% reduction in infestation among fruits treated with coconut oil. Coconut oil may be a more safe and practical choice than synthetic pesticides, since it is an edible oil with lower risk of toxicity to humans. However, we were unable to definitively pinpoint the mechanism for the effectiveness of coconut oil; this will require further research. Further study is also needed to ascertain the comparative efficacy of coconut oil for reducing fruit fly attack when applied to large red chili plants in a green house versus open field setting. Finally, in order to determine the dose and interval of application, we need to better understand how long the protective effects of coconut oil formulations persist on large red chili fruit before losing efficacy.

## CONCLUSION

In conclusion, among the 6 vegetable oil formulations tested, coconut oil was found to be the most effective against damage by *B. dorsalis* females. Fruit treated with this formulation experienced reduced fruit fly landings, oviposition, and infestation. Further research is required to definitively identify the mechanisms by which coconut oil reduces fruit fly oviposition on large red chili fruits.

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