

**EFFICACY OF EDIBLE OILS AGAINST BEAN BEETLE,
Callosobruchus maculatus (FABRICIUS) (COLEOPTERA:
CHRY SOMELIDAE: BRUCHINAE), ON STORED MUNG BEAN,
Vigna radiata (L.) R. WILCZEK**

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ABSTRACT

Edible oils were evaluated for their surface treatment, residual and post-oviposition effects, to determine their mode of action against various stages of bean beetle, *Callosobruchus maculatus*. Bioassay studies revealed that edible oils are effective seed protectants at 0.5% to 1.0% v/w dosages. Post-oviposition test showed that edible oils possess ovicidal and larvicidal properties against bean beetles which suffocate the eggs outside and larvae inside the seed after oil application. The edible oils were found effective for two months against all the stages of bean beetles. Surface treatment with edible oils can shorten the adult life span of bean beetle by preventing insect respiration resulting to suffocation. While the mode of action of edible oils is mainly a physical type of activity, any chemical toxicity or deterrence is only secondary. As a result, edible oils are more effective as seed protectants with its direct physical mode of action than volatile oils which rely on chemical toxicity or deterrence to bean beetle for control. Furthermore, investigation on the residual effectiveness of edible oils is recommended.

Keywords: *bean beetle, Callosobruchus maculatus, mungbean, Vigna radiata, oviposition deterrence, F₁ emergence, edible oils, post-oviposition, surface-treatment, residual effectiveness*



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INTRODUCTION

Stored product pest infestation is of major concern in the food and feed processing industry. In stored seeds, several primary pests are associated with various crop seeds, leguminous species in particular. According to Morallo-Rejesus and Rejesus (2001), post-harvest losses due to insects have been estimated at 5% to 50% in grains and processed products. In grains alone, millions of dollars are lost due to insect infestation in storage. Consequently, the Philippines has to import an estimated 5% to 10% for its total grain requirement (Morallo-Rejesus and Rejesus 2001). In 2020, Philippines imported \$80.3M in dried legumes, becoming the 32nd largest importer of dried legumes in the world (OEC 2020). At the same year, dried legumes were the 241st most imported product in the country. It has imported dried legumes primarily from: Indonesia (\$16.2M), Canada (\$15.6M), United States (\$14.8M), Burma (\$14.4M), and Argentina (\$6.95M) (OEC 2020).

Stored seeds are normally admixed with insecticides to prevent storage insect pest infestation (Francisco et al, 2009). This practice, however, does not conform to basic organic principles, hence, unacceptable to organic standards (Swezey et al. 2007). Organic seed with corresponding organic practices, besides formulating an effective seed protectant, would promote seed resistance against insect pest infestation (Pecenka, 2021). This then would eliminate dependence on synthetic seed treatment products, thus reliance on natural products in seed protection. Eventually, this will support genuine organic crop production in the country through the realization of organically produced seeds available to farmers.

The practice of organic seed treatment is still inadequate, despite several reports and published information of its utility, due to concerns in the use of organic products in seeds for storage. Major concerns include ineffectiveness, low persistence, high volume requirement, processing, and preparation time (Forman *et al.* 2012, Pathak 2019). The use of oil-based seed protectants is very promising considering that horticultural and mineral oils are readily available in the market (Nile *et al.* 2019). These products are used against aphids, whiteflies, and other small sized insect pests in the field, and edible oils could possibly be used in storage if properly tested (Cranshaw 2013).

Several studies have been reported on the effect of various materials such as oils against the eggs and adults of *Callosobruchus maculatus* (F.) (Soe *et al.* 2020, Bhardwaj and Verma 2012, Nisar 2022), but this is the first attempt to examine the effect of edible oils not only on eggs and adults but also on the larvae of the beetle inside the mungbean seeds. Therefore, this study was conducted to evaluate the

efficacy of edible oils against bean beetle, looking at the residual effectiveness, post-oviposition effectiveness and surface oil treatment effect against bean beetle. The residual effect and surface oil treatment were conducted to determine its effects on adult bean beetles while the post-oviposition bioassay was conducted to evaluate the effect of oils on the eggs and larvae of bean beetles.

METHODOLOGY

Seed Treatment. For each treatment, 100 g of fresh mungbean [*Vigna radiata* (L.) R. Wilczek]] seeds, cv. NSIC Mg14, were placed in a 4 in. x 12 in. polyethylene plastic bag (Calypso Plastic Center Corp., Binondo, Manila, Phil.). The edible oils used were canola and corn oils (Baguio brand, Chem Ban Yek & Co. Inc., San Juan City, Phil.), coconut oil (Minola brand, San Pablo Manufacturing Corp., San Pablo City, Phil.), olive oil (Arriba brand, Gold Bay Industries, QC, Phil), palm oil (Golden Fiesta brand, SAFI/HUFC, Ortigas Center Pasig City, Phil.), sesame oil (Pearl River brand, K-5 Industries, QC, Phil.), vegetable oil (Cook Best brand, POMS Ventures Corp., Legazpi Village, Makati City, Phil.), and virgin coconut oil (O'Mark brand, Science Park, UPLB, Los Baños, Laguna, Phil.). One percent (v/w) edible oils were added to the mungbean seeds, which were vigorously agitated to obtain an even coating of the oils on the seed coat of the beans.

Bean Beetle Rearing. Bean beetles [*Callosobruchus maculatus* (Fabr.)] obtained from the seed storage facility of BPI-Los Baños National Crop Research, Development and Production Support Center (BPI-LBNCRDPSC) were reared on mungbean seeds [*Vigna radiata* (L.) R. Wilczek]] cv. NSIC Mg14, in 34 x 24 x 10 cm plastic boxes (Big Apple Plastic Packaging, San Pedro, Laguna, Phil.) (Fatima *et al.* 2016). A kg of mungbean seeds was divided equally (500 g each) in the two rearing plastic boxes. The collected bean beetles were distributed equally in the rearing box jars with approximately 250 individuals for each sex.

For all the bioassays, the percentage of deterrent activity was determined by calculating the reduction in oviposition following the equation provided by Sadeghi *et al.* (2006):

$$OD\% = \frac{C_n - T_n}{C_n} \times 100$$

where C_n = Number of insects in control plates
 T_n = Number of insects in treated plates

Experimental Design. Three replications for each treatment in a completely randomized design (CRD) were followed for all the laboratory bioassays.

Surface Oil Treatment Using Edible Oils Against Adult Bean Beetles. Adult bean beetles were used as test insects for the surface oil treatment bioassay. Ten bean beetles in each petri dish (9 cm diameter) were used with three replications. For the treatment, 0.1 ml each of the edible oil was applied to each petri dish and distributed equally on the surface using a sterile cotton bud. The adult beetles were placed inside the surface-treated petri dishes and kept at room temperature. A negative control with nothing added on the petri dish was maintained. Beetle mortality was recorded after 6, 12, 24, 48, 72 and 96 h after beetle introduction. Data were recorded as time (h) to reach 100% beetle mortality.

Post-Oviposition Bioassay. A 250 g mungbean seed of the same cultivar, as in the previous bioassays, was exposed to bean beetle for oviposition. After 24 h of exposure, the beetles were separated from the mungbean to avoid further oviposition. The seeds were stored in a glass bottle and served as the source of eggs. For this bioassay, five mungbean seeds with ten eggs were selected and placed inside a 5 dram vial. This was repeated for all oil treatments while the negative control did not receive any treatment. Using a Medicpro 1.0 ml disposable syringe (Putting Bato, Antipolo City, Phil.), 0.03 ml oil was applied to the seeds. Oil treatment was made at 1, 5, 10, 15, 20, and 25 d after oviposition. The seeds were then observed for F_1 (first generation adult) emergence. Mean F_1 emergence were computed after all the emergences have completed or seven days after the first observed F_1 emergence. The experiment was laid out in a three-factor completely randomized design with three replications. The edible oil, dosage and time of oil application served as the main factors.

Seeds treated with oil at different days after oviposition were dissected to check for the presence of larvae and to determine the instar or stage(s) affected by the oil treatment. Ten seeds from each treatment were dissected by cracking the mungbean seeds using a small hammer. The cracked seeds were examined under a stereomicroscope for larvae/larval parts. Progeny development as affected by the oil was determined by counting the number of F_1 emergence.

Residual Effectiveness. The residual effect of different edible oils was determined by storing treated mungbean seeds in sealed bottle after edible oil application. After two months of storage, the seeds were exposed to bean beetles to measure oviposition deterrence and F_1 emergence of bean beetle. After 28 to 35 days of bean beetle exposure, the number of adult insects were recorded, analyzed, and compared

with the results obtained from the bioassay using one-week old oil treated seeds for the % oviposition deterrence (OD) and F1 emergence.

Data Analysis. Data were statistically analyzed to test for significance using ANOVA at $\alpha=0.05$ and mean separation based on Dunnett’s test.

RESULTS AND DISCUSSION

Surface Oil Treatment Using Edible Oils Against Adult Bean Beetle

Results showed that for all edible oil treatments, beetle mortality reached 100% within a shorter period compared to the negative control. The mean time (h) to attain 100% mortality for all edible oils is 80 h, which was significantly lower ($p=0.0008$) than 152 h of the control (Table 1).

Table 1
Adult bean beetle [C. maculatus (F)] mortality due to surface treatment of mungbean seeds with different edible oils (n=10)

EDIBLE OILS	ADULT BEAN BEETLE MORTALITY (%)								
	Time (h) to 100% Mortality								
	6	12	24	48	72	96	120	144	168
Olive oil	23.33	26.66	36.66	83.33	100				
Vegetable oil	13.33	33.33	40.00	76.67	100				
Canola oil	16.67	40.00	50.00	76.67	100				
Coconut oil	6.67	26.67	43.34	86.67	100				
Virgin coconut oil	6.67	16.67	36.67	80.00	96.67	100			
Palm oil	6.67	23.34	43.34	80.00	93.33	100			
Corn oil	16.67	23.34	33.34	70.00	90.00	96.67	100		
Sesame oil	10.00	20.00	23.33	63.00	93.33	96.67	96.67	100	
Control	0.00	13.33	23.33	43.33	60.00	73.33	83.33	96.67	100

The edible oils caused clogging of spiracles of the adult beetles resulting to suffocation (Don-Pedro, 1989; Obeng-Ofori, 1995; Abulude, et al. 2007). However, the possibility of affecting the bean beetles through inhalation of any substance emitted by the oil is very remote considering that the fumigating property of oils is only attributed to volatile oils (Chaubey, 2008). In the present study, it was observed that at certain levels of surface coating, the oils were still lethal to the bean beetles if they were picked up by the body of the insects and eventually covered much of their spiracles. The insect cuticle is non-polar in nature thereby attracting oily substances. The surface treatment toxicity of edible oils was attributed to the adherence of oil on the outer covering of the adult bean beetles which eventually affected the respiration

of the insects through clogging of the spiracles. This demonstrated the ability of the oils to act as clogging materials, thus preventing respiration (Obeng-Ofori, 1995). In effect, they suffocate the insect and eventually killing them. On the other hand, Stadler et al. (2002) demonstrated the softening of the cuticle in adult cotton boll weevils *Anthonomus grandis* Boh. (Coleoptera: Curculionidae) as caused by mineral oils, which implies that mineral and vegetable oils can attain a competing equilibrium with some of the components of the insect cuticle wax layer and soften the cuticle. The observed variation in cuticle hardness suggests that oils induce structural changes in the cuticle (Stadler, et al. 2002), where the correlation between cuticle softening and oil toxicity in laboratory bioassays was associated with increased mortality. This softening of the bean beetle cuticle was not measured, but in general, the bean beetles exposed to oils became less active and stayed in one place until they died.

Post-Oviposition Bioassay

The post oviposition bioassay was designed to determine the potential ovicidal and larvicidal effects of different oils on the eggs and immature bean beetles in the infested mungbean seeds. Results revealed that non-emergence of bean beetles in oil-treated mungbean seeds was significantly ($p < 0.001$) lower than those untreated mungbean seeds, and that there was no significant difference among treatments (data not shown). The emergence of one to three adults per treatment, as observed in olive, sesame, and virgin coconut oils, can be attributed to the ability of the larvae to escape contact with the oil or the oil did not reach the larvae even if it has penetrated the seeds. With the oil not being able to completely coat the seeds, both the seeds and beetles' respirations were not affected by the oil film, therefore, the insect larvae were able to complete their cycle and emerged as adult beetles. These results supported both the ovicidal and larvicidal activity of the edible oils through direct contact or blockage of seed pores that serve as respiratory pores for the larvae. The mode of action of oils on insects and its various stages is multifold, particularly on its effect on eggs, where oils can act as respiratory blocker. This demonstrates the ability of the edible oils to act as suffocating materials with the possibility of preventing respiration, as previously demonstrated by Obeng-Ofori (1995). The effect of plant oils on insect development could have been caused by physical properties of coating and blocking respiration rather than by specific chemical effect (Abulude, et al. 2007). A number of hypotheses were proposed on the ovicidal effect of oils. One of which was the observation of Don-Pedro (1989) that plant oils generally exert ovicidal action against insect pests of storage products, which was further supported by the results of Osekre, et al. (2002), that embryo mortality occurs when the oil was applied at the anterior end of the egg.

The dissections validated the results of the post-oviposition bioassay, where dead larvae of bean beetles were observed inside the mungbean seeds treated with 2.5% oil at 20 and 25 d after oviposition (Figures 1a and b). This finding suggests that the oil was able to penetrate the seeds, increased adult mortality, lowered oviposition rates and interfered with larval development. According to Swella and Mushobozy (2007), oils caused high mortality of eggs and larvae on the seed surface but had no effect on individual larvae that successfully entered the seed. This is true for low dosages (<1.0%) of oil used. But at a dosage of >1.0%, larval mortality was very high in all larval instars of the bean beetles, from the dissections conducted. This validated the assumption that the edible oils were able to penetrate the seeds and adversely affected the bean beetles by coating and suffocating them. Thus, the oil did not only exert ovicidal activity but also larvicidal activity.

Residual Effect of Edible Oils in Protecting Seeds Against Bean Beetle

The residual effect of edible oils as seed protectant against bean beetles was determined by measuring the decline or increase in activity due to prolonged storage of the treated mungbean seeds. The first bioassay served as the reference data to which succeeding bioassays were compared. Table 2 summarized the results in the free-choice bioassays separately conducted in two months interval. It demonstrated that the edible oils consistently allowed the bean beetles to oviposit their eggs on the mungbean surface but prevented the eggs/larva to develop into adult forms. Moreover, the F1 that emerged from oil-treated seeds were all significantly ($p<0.0001$) lower compared with the untreated seeds (control) for both bioassays.

Figure 1
Dead larvae of bean beetle inside the oil treated seeds at 20 (a) and 25 (b) d after oviposition

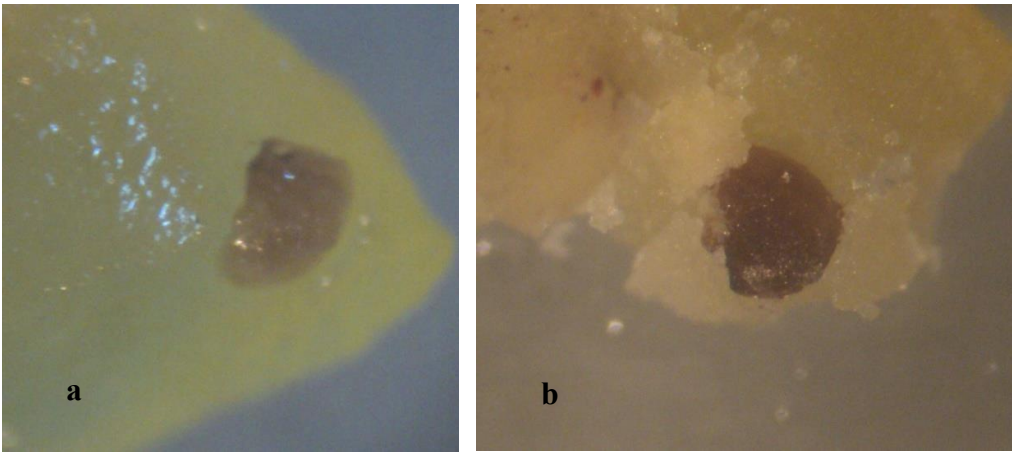


Table 2

*Effect of oil treatments on oviposition deterrence and F1 emergence of bean beetle [*C. maculatus* (F)] from 50 mungbean seeds in two separate free-choice bioassays*

EDIBLE OILS	TIME AFTER SEED TREATMENT			
	ONE WEEK		TWO MONTHS	
	Corrected Oviposition Deterrence (%)	F ₁ Emergence (%)	Corrected Oviposition Deterrence (%)	F ₁ Emergence (%)
Canola oil	32.26	0.00	32.31	0.28
Coconut oil	24.16	0.00	18.74	0.71
Corn oil	34.42	0.00	13.57	0.44
Olive oil	49.18	0.00	70.55	0.00
Palm oil	32.04	0.00	33.46	0.00
Sesame oil	24.06	0.36	0.76	0.00
Vegetable	19.41	0.00	-4.97	0.55
Virgin coconut oil	44.06	0.51	33.08	0.00
Control	0.00	65.23	0.00	58.13
cv (%)	NS	20.42	NS	49.30

No significant differences were found among treatments for each parameter

For canola oil, there was no change in its oviposition deterrence (OD) activity for having very close values for both bioassays. However, there was a slight increase of 0.28% in F1 emergence after two months. This remains very low compared to the 58% in the untreated seeds.

For coconut oil, there was a slight decrease in OD but an increase of 0.71% in F1 emergence after two months. The results indicated that the effectiveness of coconut oil as seed protectant against bean beetles slowly decline through time. But such decline is not so drastic that the bean beetles could no longer multiply in storage. This supported the observation by Doharey, et al. (1990) that coconut oil was found effective against *C. chinensis* for a storage period of six months. In addition, Busungu and Mushobozy (1991) reported that coconut oil provided the second-best protection of the natural products against cowpea bruchid. The present result also supported observations from other studies which showed that coconut oil was effective in controlling *Zabrotes subfaciatus* Boheman, a Mexican bean weevil (Busungu and Mushobozy, 1991).

Corn oil showed a decrease in OD and an increase of 0.44% in F1 emergence. The activity of corn oil might be similar to coconut oil, in which the activity declined through time could still be effective in preventing reproduction of bean beetles for longer periods. Olive oil showed an increase in OD and consistently prevented any F1 emergence. This made olive oil the best among the edible oils evaluated with the highest OD (49% after one week and 71% after two months) and consistently

provided zero F1 emergence for both bioassays. Palm oil was also found consistently having close values of OD and zero F1 emergence for both bioassays, making it the second-best edible oil tested. Sesame oil showed a decrease of 23% OD after two months, nevertheless, the F1 emergence has improved to zero. Sesame oil could still be a good seed protectant considering the zero F1 emergence indicating that the bean beetle cannot proceed to multiply even though a number of eggs have been deposited on the seeds. Vegetable oil showed a decrease of 24% in OD and an increase of 0.55% F1 emergence, making it the least effective among the edible oils as seed protectant. It provided the lowest OD for both bioassays and second to the last with the highest F1 emergence. Virgin coconut oil showed good results in terms of OD with a high value from the initial bioassay but declined by as much as 11% after two months. Also, the F1 emergence has declined to zero after two months, which is considerably more important in seed protection.

Ranking the edible oils based on its residual effectiveness as seed protectants would be as follows, from highest to lowest: olive oil > virgin coconut oil > palm oil > sesame oil > canola oil > corn oil > vegetable oil > coconut oil.

CONCLUSION

Edible oils at 0.5% to 1.0% dosage can significantly provide sufficient seed protection against bean beetle. Dosages higher than 1.0% would not be cost effective since low dosages have been shown to effectively reduce F1 emergence and arrested any population build-up. Once the oil comes in contact with the insect cuticle, it will spread through the epidermis and will clog up the insect spiracles and eventually lead to death of the insects by suffocation. The insect cuticle is non-polar in nature thereby attracting oily substances. When edible oils are applied to the seeds, they tend to coat the seed, making it greasy and unfavorable for egg oviposition. When bean beetles laid their eggs on the seeds, they would be coated with oil, and egg respiration would be obstructed. Such suffocating effect on eggs explains the ovicidal attribute of edible oils. In terms of possible effect of oils on larval and pupal mortality, the present study demonstrated that the oil which entered the seeds also coated the larvae inside the seeds. Although, no pupa was found in the dissected seeds, it was considered that the larvae died before they pupated. The toxicity of edible oils to the various stages of bean beetle may also affect the beetles and their immature forms, but only secondary relative to the physical effects exhibited by the edible oils.

RECOMMENDATION

The present study opens up new researchable areas in exploring potential utilization of edible oils both in storage and in the field. This also provided an additional venue to explore in addressing organic ways of seed protection and, furthermore, organic crop protection in general. Innovative techniques in the use of these oils must be explored to further their efficacy and avoid adverse effects on the seed. It is therefore recommended that the following be considered in future research: Effectiveness of edible oils against major storage insect pests of various seeds of economic importance; determination of the residual effect of oils for longer duration – six to one year seed storage; evaluate the combination of effective powdered organic materials with edible oils as seed protectants; and colonization and establishment of bean beetle on large quantity of oil-treated seeds in storage.

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