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Prospects of Codling Moth Management on Apples with Botanical Antifeedants and Repellents

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Abstract: The codling moth, *Cydia pomonella* L. (Tortricidae), is a major pest of apples, potentially causing annual losses exceeding USD62 billion globally. Growers have limited options for combatting the codling moth. Sprays with azinphos-methyl have been banned in the European Union, Turkey, and the USA. To be effective, *Bacillus thuringiensis* or *Carpocapsa pomonella* must be ingested in large quantities, and the fruit is damaged before the larvae die. Mating disruption or an attract-and-kill strategy does not resolve problems caused by the migration of moths from adjacent areas or insecticide resistance. Discouraging neonates from burrowing into the fruit with feeding deterrents or repellents of plant origin may become a new strategy. This paper presents a list of twenty-three plants and six secondary metabolites preventing apple infestation by codling moth neonates. Some of these plant extracts or oils (*Alium sativum*, *Tanacetum vulgare*, *Atremisia arborescens*, *Ginkgo biloba*) showed deterrence exceeding 95% in comparison to controls. The prospects of codling moth control with botanicals are discussed, and further studies on these substances are suggested. In conclusion, the author states that twenty-nine plant-originated materials have great potential in organic apple protection. Future studies should concentrate on formulating these botanicals and identifying their molecular targets.

Keywords: *Cydia pomonella*; apple protection; internal fruit feeders

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1. Codling Moth Biology and Management

The codling moth, *Cydia pomonella* L. (Tortricidae), is a major cosmopolitan pest of apples. Its current distribution includes the United States of America (USA), Canada, Chile, the British Isles, Northern and Central Europe, the Balkans, Spain, Italy, Morocco, Russia, China, Kazakhstan, Georgia, China, Australia, and New Zealand [1]. In unmanaged orchards, fruit infestation by the codling moth may reach up to 80% [2,3], which potentially translates to annual losses exceeding USD62 billion globally [4].

The mature larvae of codling moths overwinter under loose bark, in soil, or in debris around the base of trees and pupate at the time of pink bloom. At full bloom, the adults emerge and mate, and females lay eggs on leaves [3,5]. The grower has limited options to combat the codling moth: shortly after hatching from the egg, neonate larvae burrow into the fruit and stay there until their development is complete [3]. In most locations, *C. pomonella* has two generations per year. However, in the areas where the codling moth has partial or full third generations, the neonates attempt to penetrate the fruit within days of harvest, i.e., the time when insecticides are not permitted to be used, making pest control even more problematic.

Historically, sprays with the broad-spectrum organophosphate neurotoxin azinphos-methyl were a popular control measure, even though this insecticide had to be applied in excessive amounts of 1.7 kg per hectare, largely due to codling moth resistance. Exposure to azinphos-methyl has been linked to health problems for agricultural workers and aquatic ecosystems by the U.S. Environmental Protection Agency, leading to a full ban on the use of this insecticide on apples in the USA in 2013 [6]. Azinphos-methyl has also been banned in the European Union since 2006 [7] and in Turkey since 2013 [8].

Insecticides based on natural pathogens of the codling moth, such as *Bacillus thuringiensis* or *Carpocapsa pomonella*, are expensive, and, to become effective, must be ingested in large quantities. In such situations, fruit damage is often carried out before the larvae die. Pheromone-based insect-control measures such as mating disruption or attract-and-kill strategies do not resolve problems caused by the migration of moths from adjacent areas [9] or insecticide resistance [10].

Discouraging neonates from burrowing into the fruit with feeding deterrents or repellents of plant origin may become a new strategy. However, despite a recent increased interest in botanicals [11–14], papers on the prevention of fruit infestation by the codling moth with botanicals are scarce. Moreover, previous studies have used different bioassays, which makes it difficult to assess the mode of action of the botanicals tested.

In this paper, I scrutinize the methodologies used in studies examining the effects of botanicals on fruit infestation by the codling moth, and summarize the progress made in these studies to date. The prospects of using products of plant origin to prevent fruit infestation by the codling moth are discussed.

2. Bioassays Used in Experiments with Botanical Antifeedants and Repellents and Codling Moth Neonates

Four distinct bioassays were used to study the effects of plant extracts and essential oils on codling moth neonates.

Suomi et al. collected plants in the field, and extracted plant materials in chloroform, which were then evaporated and suspended in 1% aqueous Triton-X solution [15]. Apple plugs (0.8 cm in diameter) were dipped in a liquid paraffin–polyisobutylene mixture for tissue embedding (Paraplast) to coat the plug apart from the epidermis. The suspension of tested plant material was applied to the experimental plug and allowed to dry. Only one concentration (10 mg/mL) was tested. Control plugs received solvent only.

Treated plugs were placed in 9 cm petri dishes, which were modified to provide a constant flow of the air from the edge to the center of the test arena. In each arena, a plug was placed at each 90° interval. Groups of ten neonate larvae were placed in each petri dish. After 24 h, the plugs were examined for traces of feeding or the presence of larvae in the plugs.

Two experimental designs were used. For rapid screening of 25 plant extracts, codling moth neonates were exposed to the plugs treated with a given plant extract only. Separately, a control experiment was run in which only the plugs treated with 1% aqueous Triton-X were presented to the larvae. Percentages of the neonates that initiated feeding within 24 h of the experiment served as an estimation of repellent/antifeedant activity. In total, 90% of neonates fed on the control plugs treated with 1% aqueous Triton-X. Tested plant extracts were considered to have discouraged feeding in neonates if <20% of larvae fed on the treated plugs.

In follow-up experiments, selected extracts were tested. In the arena, a plug was placed at each 90° interval with alternating test and control plugs (Figure 1A). The neonates were exposed to both control and extract-treated plugs in one setting, and the larvae present in experimental (extract-treated) versus control plugs (treated with 1% aqueous Triton-X) were counted. Data were averaged and subjected to one-way analysis of variance.

Landolt et al. assessed the repellency of plant essential oils purchased from retailers [16]. To that end, a glass rod (3 mm diameter, 15 cm long) was imbedded at each end into untreated thinning apples (Figure 1B). For screening, plant essential oils were diluted in methylene chloride to 10 mg/mL concentration and applied with a fine brush to the glass rod, creating barriers at 2 cm from each apple. Five neonate codling moth larvae were placed at the middle of the glass rod and observed until 1 h elapsed. The number of times the five larvae turned around at the barriers was recorded. This procedure was repeated twice with each of the plant essential oils tested. Control tests, evaluating larval performance following applications of solvent to the glass rod, were conducted in the same manner. The four most promising oils were diluted to 0.1, 1, 10, and 100 mg/mL concentrations, and the bioassay was run in the manner described above. Data were subjected to a paired *t*-test or regression analysis.

Kovanci adapted the procedure used by Pszczolkowski and Brown to study codling moth fruit-infesting behavior [17,18]. Briefly, small apples (about 3 cm in diameter) were used. Experimental apples were treated with 50 or 100 mg/mL solution of plant essential oil or 1,8-cineol. Apples treated with distilled water served as a control. Air-dried apples (one experimental and one control) were placed in a glass dish, one larva was placed between of them, and the entire assembly was placed in a semi-translucent container with a lid (Figure 1C). The number of neonates feeding on experimental and control apples was recorded after 24 h and subjected to Fisher's exact test.

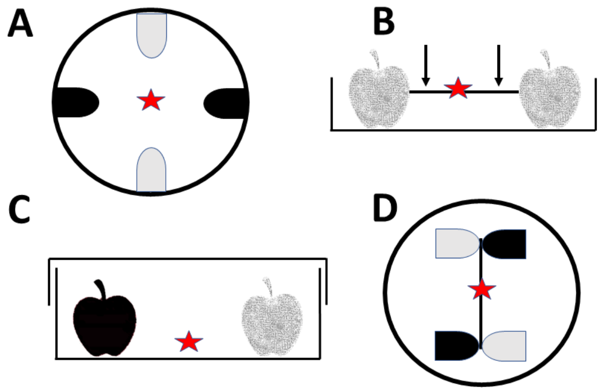

Figure 1. Schematic representation of experimental designs used in studies on preventing apple infestation by codling moth neonates with plant-derived materials. (**A**) Design of Suomi et al. [15]. Two control apple plugs (gray) and two test apple plugs (black) were placed in a Petri dish at each 90° interval with test and control plugs alternating. (**B**) Design of Landolt et al. [16]. A glass rod was imbedded at each end into untreated thinning apples (gray). Tested plant essential oils were applied to the glass rod creating barriers at 2 cm from each apple (arrows). (**C**) Design of Kovanci [17]. Two thinning apples, one test (black) and one control (gray), were placed in a glass dish and presented to codling moth neonates in a choice assay. (**D**) Design used in the laboratory of the author [19–23]. Apple plugs are arranged in pairs consisting of one test plug (black) and one control plug (gray) and connected with a glass rod, providing choice of feeding substrate for codling moth neonates. Red asterisks show the place where the neonates were released at the beginning of the tests. Remaining details are given in the text.

Another type of bioassay for feeding deterrence was used in the laboratory of the author of this article [19–23]. Here, plant tissues were air-dried, powdered, extracted with dehydration alcohol, centrifuged, evaporated, and dissolved in dehydration alcohol. The concentrations of test solutions ranged from 0.1 mg/mL to 100 mg/mL. For each dose, four plugs were taken from the same apple, using a length of plastic soda straw, in such a way that the straw covered the pulp but not the epidermis of the apple. The crevice between the plug and the edge of the straw was sealed with paraffin wax, which was applied with a warm spatula. The straws were then placed in a holder, apple plug facing up, and 5 mL of test solution was applied to each experimental plug. Dehydration alcohol was used for the treatment of control plugs. The plugs were allowed to air-dry, and four plugs were placed in a 60 mm polystyrene Petri dish, in pairs consisting of one experimental and one control plug, held in place by small pieces of modeling clay (Figure 1D). A glass rod (1.3 mm diameter, 25–27 mm long) was positioned such that each end of the rod touched both the control and the treated member of the plug pair. One neonate was placed in the middle of the glass rod, and the Petri dish was covered with a lid. The entire assembly was covered with a white plastic cupola to provide a white, slightly opaque cupola, and illuminated by fluorescent tubes. Such an arrangement provided dispersed, non-directional light over each test arena, which was important because codling moth neonates exhibit mild phototropism [24]. After 24 h, it was determined which plug had been fed upon. Data were subjected to Fisher's exact test.

3. Tested Plants and Their Effects on Codling Moth Behavior

Twenty-three plants belonging to nine families prevented apple infestation by neonates of codling moth while tested in a form of extracts or essential oils in laboratory experiments [15–17,19–22,24]. The scientific and common names of these plants are given in Table 1.

Table 1. List of plants preventing apple infestation by codling moth neonates.

Scientific Name	Family	Common Name	Reference
Alium sativum L.	Liliaceae	Garlic	[15,16]
Artemisia absinthium L.	Asteraceae	Absinthe wormwood	[15,19,20]
Artemisia annua L.	Asteraceae	Sweet wormwood	[21]
Artemisia arborescens L.	Asteraceae	Tree wormwood	[22]
Artemisia arborescens x absinthium Hancock	Asteraceae	Powis Castle wormwood	[19,20]
Artemisia ludoviciana Nutt.	Asteraceae	Silver wormwood	[19]
Citrus limon Osbeck	Rutaceae	Lemon	[16]
Elettaria cardamomum L.	Zingiberaceae	Cardamom	[17]
Ericameria nauseosa G.L. Nesom & G.I. Baird	Asteraceae	Rabbitbrush	[15]
Eucalyptus globulus Labille	Myrtaceae	Eucalyptus	[16]
Geranium viscosissimum Fisch. & C.A. Mey	Geraniaceae	Geranium	[15]
Ginkgo biloba L.	Ginkgoaceae	Ginkgo	[19,24]
Madia glomerata Hook	Asteraceae	Tarweed	[15]
Lavandula angustifolia L.	Lamiaceae	Lavender	[16]
Pinus monticola Douglas	Pinaceae	Western white pine	[15]
Pogostemon cablin Blanco	Lamiaceae	Patchouli	[16]
Ruta graveolens L.	Rutaceae	Rue	[16]
Solanum dulcamara L.	Solanaceae	Bittersweet	[15]
Tagetes glandulifera Schrank	Asteraceae	Tagetes	[16]
Tanacetum vulgare L.	Asteraceae	Tansy	[15,16]
Tropaoleum majus L.	Cruciferae	Nasturtium	[15]
Veratrum californicum Durand	Liliaceae	False Hellebore	[15]
Zingiber officinale Roscoe	Zingiberaceae	Ginger	[16]

The author categorized the effect of botanicals into strong, medium, and weak categories. Wherever numbers of codling moth neonates feeding on control and experimental fruit were presented in original reports, the percentage of fruit avoidance was adopted as a measure of the effects. Feeding deterrence at 91–100% was considered a strong effect, at 80–89% it was considered a medium effect, and lower than 80% was classified as a weak effect. Only in the work of Landolt et al. [16] was the level of statistical significance used for evaluation of the effects. Feeding deterrence was considered strong at $p < 0.001$, medium at $p < 0.02$, and weak at $p < 0.03$ and $p < 0.04$.

The following plant extracts or essential oils exhibited strong antifeedant or repellent activities: garlic, absinthe wormwood, tansy, false hellebore, ginger, patchouli, and rue (Tables 2 and 3). Cardamon showed weak deterrent or repellent activities (Table 4). Sweet wormwood, tree wormwood, and ginkgo strongly repelled codling moth neonates (Table 5).

Table 2. The results of the experiments by Suomi et al. [15]. Plant extracts were tested at one concentration of 10 mg/mL. Adapted from [15].

Plant Species	Antifeedant/Repellent Effects
Garlic	strong
Absinthe wormwood	strong
Tansy	strong
False hellebore	strong
Rabbitbrush	medium
Tarweed	medium
Western white pine	medium
Bittersweet	medium
Nasturtium	medium
Geranium	medium

Table 3. The results of the experiments by Landolt et al. [16]. Adapted from [16].

Plant Species	Concentrations Tested (mg/mL)	Repellent Effects
Garlic	0.1–100	strong
Tansy	0.1–100	strong
Ginger	10	strong
Patchouli	0.1–100	strong
Rue	0.1–100	strong
Eucalyptus	10	medium
Lavender	10	weak
Tagetes	10	weak
Lemon	10	weak

Table 4. The results of the experiments by Kovanci [17]. Adapted from [17].

Plant Species	Concentrations Tested (mg/mL)	Antifeedant or Repellent Effects
Cardamom	50–100	weak

Table 5. The results of the experiments with plant extracts by Pszczolkowski's team. Adapted from [19–22].

Plant Species	Concentrations Tested (mg/mL)	Antifeedant or Repellent Effects	Reference
Ginkgo	0.03–45	strong	[19,24]
Sweet wormwood	0.1−10	strong	[21]
Tree wormwood	0.1−10	strong	[22]
Silver wormwood	10	strong	[19]
Powis Castle wormwood	10	strong	[19]
Absinthe wormwood	0.3–10	medium	[19,20]

Medium antifeedant or repellent activities were demonstrated in extracts or essential oils from rabbitbrush, tarweed, western white pine, bittersweet, geranium, nasturtium, eucalyptus, and silver wormwood (Tables 2, 3 and 5). Lavender, tagetes, lemon, and Powis Castle wormwood had weak antifeedant or repellent activities (Tables 3 and 5).

Several secondary metabolites of plants from the *Artemisia* genus, ginkgo, and cardamom were also tested for their antifeedant and repellent activities. Only ginkgolic acid 15:0 had strong antifeedant or repellent effects (Table 6). Artemisinin, 1,8-cineole, α-thujone, bilobalide, and ginkgolide B had weak antifeedant or repellent activities (Table 6).

Table 6. The results of the experiments with plant secondary metabolites by Pszczolkowski's team. Adapted from [17,21,22,24].

Substance	Concentrations Tested (mg/mL)	Antifeedant or Repellent Effects	Reference
Ginkgolic acid 15:0	0.2–5	strong	[24]
α-thujone	1–100	weak	[22]
Artemisinin	0.1–30	weak	[21]
1,8-cineole	0.1–300	weak	[17,21]
Bilobalide	0.001–10	weak	[24]
Ginkgolide B	0.1–10	weak	[24]

Of note, the materials of plant origin were effective at relatively high concentrations ranging from 10 to 300 mg/mL. Moreover, only in the experiments by Landolt et al. did the tested materials exhibit clearly repellent activities [16]. The methodologies of the remaining experiments made it impossible to distinguish between feeding deterrence and the repellency of tested substances.

4. Prospects of Codling Moth Control with Botanicals: An Opinion

Twenty-three plant essential oils or extracts prevent apple infestation by codling moth neonates in a laboratory setting, showing some potential for using them as botanical insecticides in codling moth control. In general, botanical insecticides are very friendly to many non-target organisms [25–29]; their residues are degraded easily and rapidly through natural degradation mechanisms [30–32]. In most cases, they do not contain any substances toxic to homeothermic animals [13] and are friendly to pollinators and natural enemies of pests [29,33,34]. It is likely that the materials of plant origin that prevent apple infestation by codling moth neonates, mentioned in this paper, have similar attributes, and could be an inspiration for formulating bioinsecticides against the codling moth. Intensive studies are needed of the implementation of the knowledge about codling moth repellents and antifeedant botanicals into practice.

However, very few reports have been published about preventing fruit infestation by codling moth neonates in the field with plant extracts, essential oils, or plant secondary metabolites. The following botanicals were tested: homemade aqueous thyme extract [35]; and commercially available products: microencapsulated cardamon essential oil (Synthite Chemicals, Kerala, India) and 1,8-cineole (Graphic Scents Direct, CoverScent, Amsterdam, The Netherlands) [17], neem seed oil (unspecified Pakistani vendor) [36], garlic extract (Bioczos, Himal, Aleksandrow Lodzki, Poland) [37], and azadirachtin (NeemAzal-T/S, Biocont, Krakow, Poland) [38]. In comparison to a control, the fruit damage reduction factor reported for those field studies ranged from a value of 2.3 for thyme extract [35] to 18.9 for cardamom oil [17].

One publication also reported the antifeedant activity of azadirachtin residues offered to codling moth neonates on cubes of artificial diet [39].

The scarcity of such reports may be, in part, due to difficulties in obtaining suitable plant material or formulating the botanicals for field applications. Firstly, plants produce substances that are active ingredients of essential oils or extracts in small quantities. Consequently, large amounts of plant material are needed to isolate the active substances, and only a few plant species provide sufficiently high yields [13,40]. This limits the possibility of obtaining enough botanicals to conduct large-scale field trials. On the one hand, the scarce reports of field applications of plant extracts or essential oils indicate that high concentrations of botanicals must be used to effectively prevent fruit damage by codling moths [17,35–38]. It is noteworthy that artemisinin, which prevents apple infestation by codling moth neonates (Table 5), can be produced naturally in large quantities due to genetic modifications of *Artemisia annua* and even other plants [41]. Secondly, ensuring the sufficient persistence, quality, and stability of the botanicals is also a challenge. However, formulations based on micro- and nano-particles seem to improve the efficacy, persistence, and controlled release of the botanical insecticides [42–44]. In recent experiments, microencapsulated cardamom essential oil applied at 50 and 100 g/L lowered infestation ratios of apples from almost 80% to less than 2% [17]. It is likely that, with time, more plant-based products will become available for scientists and growers interested in controlling codling moth populations.

On the other hand, there is still a need for laboratory experiments with botanical insecticides against codling moth. For instance, more codling moth feeding deterrent or repellent constituents of botanicals should be identified. In 2016, nine commercial botanicals were used in California (neem oil, chenopidium, pyrethrins, azadirachtin, garlic, orange oil, geraniol, sabadilla, and capsicum) [14] but only three (neem, azadirachtin, and garlic) have been tested for the prevention of fruit infestation or inhibition of feeding by codling moths [36–39].

Studies on the molecular targets of these botanicals are also needed. Gamma-aminobutyric acid (GABA) receptors are involved in insect taste perception [45]. GABA antagonists and allosteric modulators act as antifeedants. For instance, picrotoxinin, a GABA-antagonist, and thymol (an aromatic component of thyme), an allosteric modulator (inhibitor) for insect GABA receptors, both have antifeedant properties in the lepidopteran *Spodoptera littoralis* and the coleopteran *Leptinotarsa decemlineata* [46].

If we are to see botanical feeding deterrents in widespread use, the codling moth will, eventually, develop resistance to botanical insecticides. Without sufficient knowledge about the molecular targets of the botanicals, rational planning of insecticide rotation will be impossible. Fortunately, IRAC Mode of Action Classification Scheme lists only seven pesticides that have a mode of action based on interference with GABA receptors [47]. None are registered for use on commodities that codling moths are known to attack.

Another avenue of research could be exploring possibilities of apple genetic modification in a way that active ingredients of plant extracts or essential oils known to prevent fruit infestation could be expressed in apple, in amounts making the fruit unpalatable to codling moth larvae, but still acceptable for consumers. Some of the repellents, such as ginkgolic acids, are nonpolar compounds, and could be expressed in apple waxes capable of being removed before the fruit reaches the consumer, as it is currently carried out [24].

Finally, the bioassay methodology should be regulated. The assay by Landolt et al. [16] provides an investigation into repellence only. Using this experimental design, feeding deterrence cannot be determined since the larvae do not have direct contact with the fruit. Therefore, it should not be used in future studies. The remaining bioassays should be standardized. First, the codling moth neonates should be tested individually to avoid any possible social interactions which could influence the process of test fruit choice. Secondly, apple plugs should be used. Procuring plugs from the same fruit allows consistency to be maintained between the plugs in terms of coloration. It is known that certain colors attract codling moth neonates more than the others [48]. Thirdly, codling moth neonates exhibit phototropism [24], and therefore test areas should be illuminated with dispersed light. Only the methodology of Pszczolkowski et al. [19–23] met all the aforementioned conditions.

5. Conclusions

Papers on prevention of fruit infestation by codling moth neonates with botanicals are scarce. However, the amount of background information contained in these papers is abundant. There are twenty-three plants and six synthetic or extractable plant secondary metabolites that could potentially be used in organic apple protection. These botanicals will likely make inroads into sectors where large-scale growing is not the first priority, and a premium is placed on the safety of humans and the environment, namely, small organic farmers and amateur gardeners. At present, however, studies should concentrate on the formulation of the botanicals to enhance their bioactivity, efficacy, and longevity in the field, as well as on identifying the molecular targets of particular botanicals.

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