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# Extract of *Nicotiana tabacum* as a potential control agent of *Grapholita molesta* (Lepidoptera: Tortricidae)

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## Abstract

Oriental fruit moth, *Grapholita molesta* (Busck) (Lepidoptera: Tortricidae), is an important pest of stone and pome fruits. Growers usually depend on chemical insecticides to control this pest, but demand for more environmentally-friendly means of controlling pests is increasing. At least 91 plant extracts have been reported to be effective against other lepidopterans, but their acute toxicity against *G. molesta* has rarely been studied. Among these 91 materials, we assessed the residual toxicity of 32 extracts against first instar larvae (< 5 h old) of *G. molesta* in the laboratory. *Nicotiana tabacum* L., used at the concentration of 2 mg/ml, showed the highest corrected mortality (92.0%) with a lethal time ($LT_{50}$) value of 12.9 h. The extract was followed in its efficacy by *Allium sativum* L. (88.0%), *Zanthoxylum piperitum* (L.) De Candolle (70.0%), and *Sapindus mukorossi* Gaertner (65.0%), when mortality was assessed at 20 h after exposure. Against adult fruit moths (< 5 d old), *N. tabacum* also showed the highest corrected mortality among tested extracts, being 85 and 100% in adult females and males, respectively, at 168 h after exposure. However, there was no synergistic effect of the combined application of any of the top four extracts in either laboratory or greenhouse assays. Oviposition by *G. molesta* on peach twigs was reduced 85–90% when *N. tabacum* was applied at 4 ml/ twig compared to control (methanol), demonstrating that *N. tabacum* may have potential for use as a botanical insecticide against *G. molesta*.

## Introduction

Oriental fruit moth, *Grapholita molesta* (Busck) (Lepidoptera: Tortricidae), is a serious pest of fruit trees in the temperate regions, worldwide [1–4]. Its host range encompasses species within the family Rosaceae, mostly those from the genera *Prunus* and *Pyrus* [1]. Stone fruit peach [*Prunus persica* L. (Rosales: Rosaceae)] is considered the primary host of *G. molesta* whereas the pome fruits pear [*Pyrus communis* L. (Rosales: Rosaceae)] and apple [*Malus domestica* L. (Rosales: Rosaceae)] are considered secondary hosts [5].

Application of organophosphorus, carbamates, or synthetic pyrethroid pesticides is a common method for control of *G. molesta* in Korea [6, 7], but the development of insecticide

resistance is a serious threat to the fruit industry [6], and *G. molesta* has developed resistance to 14 insecticides including 10 organophosphates [8]. As many of these insecticides are neurotoxins, they have some potential to be harmful to non-target organisms, including people and domestic animals [4]. To avoid such risks, new pest management tactics need to be developed for the management of *G. molesta*. Due to their less residual toxicity, lower development cost, and general safety to people, plant extracts have the potential to be effective alternatives for control of pest insects [9].

Secondary plant metabolites, such as polyphenols, terpenoids, alkaloids, steroids, lignans, essential oils, fatty acids, and sugars, are regarded as defense mechanisms against insect attack [10]. Some secondary metabolites inhibit insect development and reproduction, while others act as antifeedants, repellents, or fumigants [11–13]. Botanical insecticides degrade quickly, meaning their impact on beneficial or non-target organisms is less than that of conventional insecticides [14], thus would be more compatible with biological control agents than synthetic insecticides. Furthermore, botanical insecticides have also multiple modes of action, development of resistance in insects has been reported less frequently [15].

At least 91 plant extracts have been found effective against pest lepidopterans in studies published from 2000–2015 (Table 1). Some of these extracts have demonstrated a similar level of pest toxicity as synthetic insecticides. Extracts from goat weed (*Ageratum conyzoides* L.) and siam seed (*Chromolaena odorata* [L.]) controlled *Plutella xylostella* L. larvae, a rate similar to the synthetic insecticide emamectin benzoate [16]. Antifeedant activity was found for extracts of *Chrysanthemum* sp. and *Achillea millefolium* L. against *Spodoptera littoralis* (Boisduval) and *Pieris rapae* L., respectively [17, 18], and plant extracts have also been found to act as an oviposition deterrent; Reegan et al. [19] reported that a hexane extract of *Limonia acidissima* (L.) showed 100% oviposition deterrency for adults females of *Culex quinquefasciatus* Say and *Aedes aegypti* L.

As botanical insecticides are a potential alternative to conventional insecticides [9], the present study was conducted to assess the efficacy of various plant extracts against *G. molesta*. Among the 91 plant extracts reported in the literature, we could obtain only 32 plant extracts available and measured their acute toxicities against first instar larva and adults of *G. molesta*. We also evaluated the deterrent effect of these plant extracts on the oviposition of *G. molesta* females in the laboratory and under semi-field condition.

## Materials and methods

### Insect rearing procedures

Apples infested with oriental fruit moth were collected and kept in ventilated plastic containers (24.0 L × 17.0 W × 8.0 H cm) at 24.9 ± 0.1˚C, 50.2 ± 1.3% RH, and a 16:8 h (L:D) photoperiod in an incubator (DS-11BPL, Dasol Scientific Co. Ltd, Hwaseong, Republic of Korea). When the larvae reached the fifth instar, they emerged from the apple and built their cocoons in the paper towel provided for pupation. Pupae were collected and held in breeding dishes (10.0 D × 4.0 H cm, 310102, SPL, Pocheon, Republic of Korea). When adult moths emerged, they were transferred into ventilated acrylic cylinders (25.5 H × 8.5 D cm), and provided with a piece of cotton soaked in 10% sugar solution as a food source. The acrylic cylinders were kept in a desiccator (36.0 L × 28.0 W × 25.0 H cm) and incubated at 25.6 ± 0.1˚C and 91.2 ± 0.1% RH. When moths started to lay eggs on the wall, the cylinder was changed daily to collect freshly laid eggs. Acrylic cylinders bearing eggs on the walls were kept in a separate incubator at 25.6 ± 0.1˚C and 91.2 ± 0.1% RH until egg hatch, after which first instar larvae were collected for the experiments or reuse in mass rearing.

**Table 1. Plant extracts reported during 2000–2015 to show toxicity against lepidopteran insects.**

| Plant species | Plant parts | Solvent | Lepidopteran insects tested | |
|---|---|---|---|---|
| | | | Species | Family |
| *Abrus precatorius* [38] | Seed | Ethanol | *Galleria mellonella* | Pyralidae |
| *Achillea millefolium* [18] | Leaf | Methanol | *Pieris rapae* | Pieridae |
| *Acorus calamus* [39] | Rhizome | Ether | *Sitotroga cerealella* | Gelechiidae |
| *Ageratum conyzoides* [16] | Leaf | Detergent | *Plutella xylostella* | Yponomeutidae |
| *Allium cepa* [40] | Fresh onion | Tween 20 | *Tuta absoluta* | Gelechiidae |
| *Allium sativum* [40] | Fresh garlic | Tween 20 | *Tuta absoluta* | Gelechiidae |
| *Alpinia galanga* [41] | Rhizome | Ethanol | *Plutella xylostella* | Yponomeutidae |
| *Anona coriacea* [42] | Leaf | Methanol | *Spodoptera frugiperda* | Noctuidae |
| *Anona dioica* [42] | Leaf | Methanol | *Spodoptera frugiperda* | Noctuidae |
| *Anona muricata* [43] | Leaf | Ethanol | *Plutella xylostella* | Yponomeutidae |
| *Artemisia annua* [18] | Leaf | Methanol | *Pieris rapae* | Pieridae |
| *Artemisia vulgaris* [44] | Whole plant | Methanol | *Spodoptera littoralis* | Noctuidae |
| *Avicennia marina* [45] | Aerial part | Hexane | *Phthorimaea operculella* | Gelechiidae |
| *Azadirachta indica* [46] | Seed | Water | *Tuta absoluta* | Gelechiidae |
| *Bifora radiens* [47] | Whole plant | Acetone | *Thaumetopoea solitaria* | Thaumetopoeidae |
| *Cabralea canjerana* [48] | Seed/ Fruit | Ethanol | *Spodoptera frugiperda* | Noctuidae |
| *Capparis aegyptia* [45] | Aerial part | Hexane | *Phthorimaea operculella* | Gelechiidae |
| *Capsicum annum* [49] | Leaf | Methyl. chloride | *Spodoptera littoralis* | Noctuidae |
| *Capsicum frutescens* [16] | Fruit | Detergent | *Plutella xylostella* | Yponomeutidae |
| *Carica papaya* [50] | Seed | Methanol | *Spodoptera frugiperda* | Noctuidae |
| *Cassia sophera* [16] | Leaf | Detergent | *Plutella xylostella* | Yponomeutidae |
| *Chromolaena chaseae* [51] | Leaf | Ethanol | *Spodoptera frugiperda* | Noctuidae |
| *Chromolaena odorata* [16] | Leaf | Detergent | *Plutella xylostella* | Yponomeutidae |
| *Chrysanthemum grandiflorum* [17] | Aerial part | Metanol | *Spodoptera littoralis* | Noctuidae |
| *Chrysanthemum indicum* [52] | Leaf | Water | *Plecoptera reflexa* | Noctuidae |
| *Chrysanthemum macrotum* [17] | Aerial part | Methanol | *Spodoptera littoralis* | Noctuidae |
| *Chrysanthemum morifolium* [53] | Leaf | Methanol | *Trichoplusia ni* | Noctuidae |
| *Chrysanthemum segetum* [17] | Aerial part | Methanol | *Spodoptera littoralis* | Noctuidae |
| *Citrullus colosynthis* [54] | Seed | Ammonium sulfate | *Ectomyelois ceratoniae* | Pyralidae |
| *Citrus sinensis* [55] | Leaf | Phenol | *Phyllocnistis citrella* | Gracillariidae |
| *Cleome deoserifolia* [44] | Aerial part | Ethanol | *Phthorimaea operculella* | Gelechiidae |
| *Cleome spinosa* [56] | leaves | Ethanol | *Pieris rapae* | Pieridae |
| *Commiphora molmol* [57] | Stem | Water | *Spodoptera littoralis* | Noctuidae |
| *Croton urucurana* [58] | Stem | Methanol | *Anagasta kuehniella* | Pyralidae |
| *Cymbopogon martinii* [59] | Whole part | Water | *Euprosterna elaeasa* | Limacodidae |
| *Cyprus rotundus* [41] | Tuber | Ethanol | *Plutella xylostella* | Yponomeutidae |
| *Datura metel* [60] | Leaf | Methanol | *Helicoverpa armigera* | Noctuidae |
| *Delphinium consolida* [44] | Whole plant | Methanol | *Spodoptera littoralis* | Noctuidae |
| *Dimorphandra mollis* [61] | Leaf | Ethanol | *Sitotroga cerealella* | Gelechiidae |
| *Euphorbia lathyrus* [62] | Seed | Ethanol | *Spodoptera littoralis* | Noctuidae |
| *Fumaria officinalis* [47] | Whole plant | Acetone | *Thaumetopoea solitaria* | Thaumetopoeidae |
| *Ginkgo biloba* [63] | Seed coat | Methanol | *Spodoptera exigua* | Noctuidae |
| *Glycine max* [64] | Leaf | Isooctane | *Heliothis zea* | Noctuidae |
| *Gomphrena globosa* [41] | Seed | Ethanol | *Plutella xylostella* | Yponomeutidae |
| *Hordium sativum* [38] | Seed | Ethanol | *Galleria mellonella* | Pyralidae |
| *Hovenia dulcis* [65] | Leaf | Water | *Anticarsia gemmatalis* | Erebidae |

(*Continued*)

**Table 1.** (Continued)

| Plant species | Plant parts | Solvent | Lepidopteran insects tested | |
|---|---|---|---|---|
| | | | Species | Family |
| *Humulus lupulus* [47] | Whole plant | Methanol | *Thaumetopoea solitaria* | Thaumetopoeidae |
| *Hymenoxys robusta* [66] | Leaf | Methanol | *Spodoptera exigua* | Noctuidae |
| *Ipomoea pauciflora* [67] | Seed | Hexane | *Spodoptera frugiperda* | Noctuidae |
| *Jatropha curcas* [16] | Leaf | Detergent | *Plutella xylostella* | Yponomeutidae |
| *Jatropha gossypifolia* [68] | Leaf | Ethanol | *Spodoptera frugiperda* | Noctuidae |
| *Laurus nobilis* [38] | Seed | Ethanol | *Galleria mellonella* | Pyralidae |
| *Lepidaploa lilacina* [51] | Leaf | Ethanol | *Spodoptera frugiperda* | Noctuidae |
| *Lychnophora ericoides* [51] | Leaf | Ethanol | *Spodoptera frugiperda* | Noctuidae |
| *Lychnophora ramosissima* [51] | Leaf | Ethanol | *Spodoptera frugiperda* | Noctuidae |
| *Melia azedarach* [68] | Leaf | Ethanol | *Spodoptera frugiperda* | Noctuidae |
| *Millettia ferruginea* [69] | Seed | Water | *Busseola fusca* | Noctuidae |
| *Momordica charantia* [70] | Leaf | Methanol | *Leucoptera coffeella* | Lyonetiidae |
| *Nerium indicum* [71] | Seed | Water | *Helicoverpa assulta* | Noctuidae |
| *Nicotiana tabacum* [16] | Leaf | Detergent | *Plutella xylostella* | Yponomeutidae |
| *Ocimum gratissimum* [16] | Leaf | Detergent | *Plutella xylostella* | Yponomeutidae |
| *Pachyrhizus erosus* [72] | Seed | Methanol | *Plutella xylostella* | Yponomeutidae |
| *Peganum harmala* [73] | Leaf | Methanol | *Spodoptera exigua* | Noctuidae |
| *Pelargonium zonale* [40] | Leaf | Tween 20 | *Tuta absoluta* | Gelechiidae |
| *Petroselium sativum* [38] | Seed | Ethanol | *Galleria mellonella* | Pyralidae |
| *Peumus boldus* [74] | Leaf | Water | *Spodoptera frugiperda* | Noctuidae |
| *Piper amalago* [75] | Leaf | Ethanol | *Tuta absoluta* | Gelechiidae |
| *Piper glabratum* [75] | Leaf | Ethanol | *Tuta absoluta* | Gelechiidae |
| *Piper mikanianum* [75] | Leaf | Ethanol | *Tuta absoluta* | Gelechiidae |
| *Plantago lanceolata* [70] | Leaf | Methanol | *Leucoptera coffeella* | Lyonetiidae |
| *Plantago psyllium* [38] | Seed | Ethanol | *Galleria mellonella* | Pyralidae |
| *Pongamia pinnata* [76] | Seed | Chloroform | *Earias Vittella* | Noctuidae |
| *Psychotria goyazensis* [77] | Leaf | Ethanol | *Spodoptera frugiperda* | Noctuidae |
| *Psychotria prunifolia* [61] | Leaf | Ethanol | *Sitotroga cerealella* | Gelechiidae |
| *Quassia amara* [78] | Wood | Methanol | *Hypsipyla grandella* | Pyralidae |
| *Ricinus communis* [79] | Leaf | Hexane | *Spodoptera frugiperda* | Noctuidae |
| *Rhododendron molle* [80] | Flower | Ethyl acetate | *Hypsipyla grandella* | Pyralidae |
| *Ruta chalepensis* [81] | Leaf | Hexane | *Hypsipyla grandella* | Pyralidae |
| *Sapindus mukorossi* [82] | Fruit | Water | *Thysanoplusia orichalcea* | Noctuidae |
| *Siphoneugena densiflora* [83] | Leaf | Methanol | *Spodoptera frugiperda* | Noctuidae |
| *Synedrella nodiflora* [19] | Leaf | Detergent | *Plutella xylostella* | Yponomeutidae |
| *Tagetes erecta* [84] | Leaf | Ethanol | *Spodoptera frugiperda* | Noctuidae |
| *Tanacetum mucroniferum* [44] | Whole plant | Methanol | *Spodoptera littoralis* | Noctuidae |
| *Tanacetum zahlbruckneri* [85] | Flower | Methanol | *Spodoptera littoralis* | Noctuidae |
| *Tithonia diversifolia* [61] | Leaf | Ethanol | *Sitotroga cerealella* | Gelechiidae |
| *Trichilia pallens* [86] | Twig | Water | *Spodoptera frugiperda* | Noctuidae |
| *Trichilia pallida* [86] | Twig | Water | *Spodoptera frugiperda* | Noctuidae |
| *Trichogonia villosa* [51] | Leaf | Ethanol | *Spodoptera frugiperda* | Noctuidae |
| *Vernonia holosenicea* [51] | Leaf | Ethanol | *Spodoptera frugiperda* | Noctuidae |
| *Zanthoxylum limonella* [87] | Bark | Ethyl acetate | *Spodoptera litrura* | Noctuidae |
| *Zea diploperennis* [88] | Leaf | Water | *Spodoptera frugiperda* | Noctuidae |

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**Table 2. Thirty-two plant extracts evaluated in this study.**

| Plants (Reference number) | Extracted part | Family name | Plants (Reference number) | Extracted part | Family name |
|---|---|---|---|---|---|
| *Gomphrena globosa* L. (036–080) | Whole plant | Amaranthaceae | *Ginkgo biloba* L. (031–069) | Leaf-stem | Ginkgoaceae |
| *Allium cepa* L. (034-064) | Whole plant | Amaryllidaceae | *Piper Kadzura* Ohwi (001–223) | Leaf | Piperaceae |
| *Allium sativum* L. (033–033) | Whole plant | Amaryllidaceae | *Plantago lanceolata* L. (020-084) | Whole plant | Plantaginaceae |
| *Artemisia annua* L. (008–007) | Leaf | Amaryllidaceae | *Cymbopogon tortilis* J. Presl (010–002) | Whole plant | Poaceae |
| *Nerium indicum* L. (018–097) | Leaf | Apocynaceae | *Delphinium maackianum* Regel (012–093) | Whole plant | Ranunculaceae |
| *Chrysanthemum boreale* Makino (004–039) | Whole plant | Asteraceae | *Hovenia dulcis* Thunberg (015–094) | Stem-bark | Rhamnaceae |
| *Chrysanthemum coronarium* L. (034–061) | Whole plant | Asteraceae | *Citrus unshiu* Marc (018-017) | Leaf-stem | Rutaceae |
| *Chrysanthemum indicum* L. (011–005) | Whole plant | Asteraceae | *Zanthoxylum piperitum* (L.) De Candolle (011–088) | Leaf | Rutaceae |
| *Chrysanthemum morifolium* Ramat (032–009) | Whole plant | Asteraceae | *Sapindus mukorossi* Gaertner (021–040) | Leaf-stem | Sapindaceae |
| *Tagetes erecta* L. (035-092) | Whole plant | Asteraceae | *Capsicum annum* L. (026-010) | Leaf-stem | Solanaceae |
| *Humulus japonicus* Siebold & Zucc. (008–095) | Leaf-stem | Cannabaceae | *Datura metel* L. (037-098) | Aerial part | Solanaceae |
| *Cleome spinosa* Jacquin (033-098) | Aerial part | Cleomaceae | *Nicotiana tabacum* L. (036–022) | Leaf-stem | Solanaceae |
| *Citrullus vulgaris* Schrader (035–064) | Whole plant | Cucurbitaceae | *Alnus japonica* Thunberg (003–084) | Leaf | Betulaceae |
| *Momordica charantia* L. (034–065) | Whole plant | Cucurbitaceae | *Arisaema takeshimense* Nakai (001–136) | Leaf | Araceae |
| *Rhododendron micranthum* Turcz (003–023) | Leaf-stem | Ericaceae | *Xylosma congestum* (Lour.) Merrill (001–113) | Leaf | Flacourtiaceae |
| *Ricinus communis* L. (018–093) | Leaf | Euphorbeaceae | *Acer takeshimense* Nakai (001–128) | Leaf | Aceraceae |

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## Extract preparation

Methanol extracts of test plants were purchased from KPEB (Korea Plant Extract Bank, Cheongju, Republic of Korea) (Table 2). Extraction consisted of extraction, filtering and yield testing, concentration, drying, and storage (http://extract.kribb.re.kr).

## Laboratory bioassay

**Evaluation of single plant extracts.** Commercially produced plant extracts were diluted in our laboratory using methanol (99.5%, Daejung Chemicals and Metals Co. Ltd., Siheung, Republic of Korea) to make a 2 mg/ml stock solution. First instar (< 5 h old) larvae and adult male or female moths (3–5 d old) of *G. molesta* were used in our bioassays. Sex of adults used in bioassays was determined at the pupal stage by confirming the presence of an additional posterior abdominal segment in males [20]. Bioassays consisted of exposure of target life stage to an extract in scintillation glass vials (20 ml), to which 100 μl of each plant extract's stock solution has been applied and allowed to air-dry, with rotation, for 2.5 h before the assay. This process allowed the methanol to fully evaporate, leaving the plant extract as a residue on the inner surface of the vial, after which five first instar (< 5 h old) larvae or adults were place in each vial. The vials were kept in the desiccators at 25.3 ± 0.03˚C and 70.2 ± 0.8% RH for larvae and 25.2 ± 0.02˚C and 70.5 ± 0.9% RH for adults in the incubator. Methanol was used as a negative control and the synthetic insecticide λ-cyhalothrin as a positive control. Mortality was observed every 4 and 24 h for larvae and adult, respectively, until death of all insects in the negative control. Bioassays were conducted with 30 larvae and 30 adults per treatment with six replications (5 insects/ replication).

**Tests with mixed extracts.** The synergistic effects of mixtures of pairs of plant extracts were determined by the co-toxicity coefficient (CTC) method in the laboratory [21, 22]. The mixture of two plant extracts, at a 1:1 ratio and concentration of 2 mg/ml, was applied to larvae

and adults of *G. molesta*. Bioassays were conducted in glass scintillation vials similar to those described in the previous section.

Calculation of co-toxicity coefficients Sun and Johnson [21].

We calculated the co-toxicity coefficients of extract mixtures as per Sun and Johnson [21]: Co-toxicity coefficient (CTC) = ($LT_{50}$ of toxicant alone / $LT_{50}$ of toxicant in the mixture) × 100 (CTC = 100, similar action; CTC >100, synergistic action; CTC<100, antagonism).

## Greenhouse bioassay

Plant extracts were also evaluated in greenhouse trials. Before the experiment, transparent film (O.H.P film, 210 mm × 297 mm, PP2910, 3M, Seoul, Republic of Korea) was put inside the acrylic cage used for adult moths as an oviposition substrate. Eggs of this film were then collected and used for experiments. After spraying 4 ml of a given plant extract (at a concentration of 2 mg/ml) on each twig of a potted peach tree, 25 eggs were attached to five twigs (5 eggs/twig) for each treatment. Tangle trap (Tanglefoot Company, Grand Rapids, Michigan, USA) was applied at the bottom of the twig to prevent hatched larva from escaping. After 7 d, twig infestation rates were determined.

## Assessment of oviposition deterrence in laboratory assay

Oviposition deterrence effects of plant extracts were evaluated in the laboratory. Tests were carried out using peach tree twigs with five leaves each. At first, twigs (length of 10–12 cm) were put in conical flask (250 ml) filled with water to keep the twigs alive for about 7 d. Then, 4 ml of plant extracts were sprayed at a concentration of 2 mg/ml on the twigs, after which twigs were kept for 2.5 h to allow the plant extract to dry or 5 h to allow the positive control of λ-cyhalothrin to dry. Twigs in the conical flask were then placed on plastic trays and covered with ventilated acrylic cylinder cages (25.5 H × 8.5 D cm). Five mated female moths that had begun to lay eggs the previous day, together with five males, were released into each acrylic cylinder cage and held at 25.4 ± 0.1˚C, 42.1 ± 0.4% RH, and a 16:8 h (L:D) photoperiod in the growth chamber. We then observed the number of eggs laid on each twig or on the wall of a cage every 24 h for up to five days. The experiments were replicated two times.

## Assessment of oviposition deterrence in a greenhouse assay

The oviposition deterrence of plant extracts was also evaluated under greenhouse conditions. Four ml of each plant extract were sprayed onto potted peach plants at a concentration of 2 mg/ml and plants were then allowed to dry for 2.5 h. After fully drying, plants were covered with a pipe framed cage (47.0 L × 47.0 W × 115.0 L cm) screened with white-colored nylon fabric Then five female moths (mated and started oviposition one day before) and five males were released inside the cage. We then observed the number of eggs laid on each twig or on the wall of a cage every 24 h for up to five days. The experiments were replicated two times.

## HPLC analysis

**Instrumentation.** An Agilent 1200 series (Agilent, Santa Clara, CA) HPLC system was equipped with bin pump (G1312A), degasser (G13796), column oven (250 × 4.6 mm and 5 μm particle size, Agilent, Santa Clara, CA), and diode array detector (G1315B). Agilent ChemStation software was used for data acquisition and system suitability calculations.

**Chromatographic parameters.** Reverse phase high performance liquid chromatography (RP-HPLC) was used for the analysis for *N. tabacum* and *A. sativum* extract according to the method described by Tanbwekar et al. [23] with a minor modification. In our study, column

temperature was used at 25˚C instead of 35˚C. Column was used with flow rate of 1 ml/minute. Diode array detector in range of 200–800 nm was used for determining peak purity. Injection volume was 20 μl where phosphate buffer (pH 6.8; 10nm) with methanol (35.65% v/v) was used as mobile phase.

### Statistical analysis

Larval mortality data were corrected using Abbott's formula [24] and then were used to calculate the lethal median time ($LT_{50}$) using SAS 9.4 software [25]. Infestation of twigs in greenhouse and number of eggs laid on substrates in the oviposition deterrence experiment in the laboratory were analyzed using a Chi-square test with a post-hoc multiple comparison test analogous to Tukey's test [26].

In the oviposition deterrence experiment in the greenhouse, the number of eggs was analyzed using single factor analysis of variance (ANOVA) and differences in the mean number of eggs were determined by Tukey's test using Proc MIXED of SAS 9.4 [25]. Before analysis, normality and homogeneity were tested using a Kolmogorov-Smirnov test ($P = 0.150$) and a Levene test ($P = 0.442$).

## Results

### Laboratory bioassay

**Evaluation of single plant extracts.** Among the 32 plant extracts tested, *Nicotiana tabacum* L., *Allium sativum* L., and *Zanthoxylum piperitum* (L.) De Candolle showed the highest mortality on first instar larva (Table 3). The $LT_{50}$ values of *N. tabacum*, *A. sativum*, and *Z. piperitum* were 12.9 h ($\chi^2 = 9.99$, $df = 4$, $P = 0.041$), 15.6 h ($\chi^2 = 4.02$, $df = 4$, $P = 0.403$), and 16.1 h ($\chi^2 = 17.02$, $df = 4$, $P = 0.002$), respectively. The $LT_{50}$ value of *Sapindus mukorossi* Gaertner was 17.5 h ($\chi^2 = 10.04$, $df = 5$, $P = 0.074$), which was significantly higher than *N. tabacum* or *A. sativum*. *Nicotiana tabacum* showed highest corrected mortality of 92.0% followed by *A. sativum* (88.0%), *Z. piperitum* (70.4%), and *S. mukorossi* (65.2%) within 20 h (Fig 1). For the positive control, λ-cyhalothrin, 100% corrected mortality was found within 12 hours. On the basis of the $LT_{50}$ value, *N. tabacum*, *A. sativum*, *Z. piperitum*, and *S. mukorossi* were chosen as the four most effective plant extracts against first instar larvae of *G. molesta*, and these extracts were further evaluated in subsequent experiments.

In the adult assay, 100 and 96.7% of adult males survived 24 and 48 h, respectively, but only 30.0% of adult males survived 144 h when held in vials treated with *N. tabacum* (Fig 2). *Allium sativum* and *N. tabacum* both caused higher mortality than *S. mukorossi* and methanol on adult males with $LT_{50}$ values of 107.5 ($\chi^2 = 3.08$, $df = 6$, $P = 0.799$) and 109.9 h ($\chi^2 = 7.46$, $df = 5$, $P = 0.189$), respectively (Table 4). In case of adult females, *N. tabacum* and *A. sativum* were also significantly more effective than other plant extracts, with $LT_{50}$ values of 131.9 ($\chi^2 = 14.39$, $df = 6$, $P = 0.026$) and 158.3 h ($\chi^2 = 5.96$, $df = 7$, $P = 0.544$), respectively (Table 4). Irrespective of treatments, adult male *G. molesta* adult died faster than females (Fig 2).

**Evaluation of mixed extracts.** We also evaluated the effect of mixtures of plant extracts on first instar larvae (< 5 h old) and on both male and female adults (< 5 d old) of *G. molesta*. The first instar larvae of *G. molesta* died faster when treated with the mixture of *N. tabacum* +*Z. piperitum*, with corrected mortality of 90.5% at 20 h after treatment (Fig 3). The $LT_{50}$ value of the mixture of *N. tabacum*+*Z. piperitum* was 14.3 h ($\chi^2 = 11.32$, $df = 4$, $P = 0.023$), but the co-toxicity coefficient value was 90.5 indicating that there was no synergistic effect of the mixture of *N. tabacum*+*Z. piperitum*. The lethal median time ($LT_{50}$) was 76.7 h ($\chi^2 = 2.87$, $df = 4$, $P = 0.579$) for adult males, significantly different from the mixture of *N. tabacum*+*A. sativum* (Table 5) in which all adults died within 144 h (Fig 4). The co-toxicity coefficient value of *N.*

**Table 3. Statistical comparison of methanolic plant extracts (200μg/vial) against the 1st instar larva of *Grapholita molesta* by scintillation glass vial assay.**

| Treatment | $LT_{50}$ | 95% C.I | Slope ± SE | $\chi^2$ (df) |
|---|---|---|---|---|
| λ-cyhalothrin | 5.32a | 4.92–5.72 | 6.21 ± 0.58 | 2.35 |
| *Nicotiana tabacum* | 12.92b | 11.57–14.14 | 9.07 ± 1.09 | 9.99 (4) |
| *Allium sativum* | 15.57c | 15.03–16.09 | 11.16 ± 0.88 | 4.02 (4) |
| *Zanthoxylum piperitum* | 16.09bcd | 14.07–18.15 | 8.57 ± 1.40 | 17.02 (4) |
| *Sapindus mukorossi* | 17.48d | 16.32–18.62 | 9.74 ± 0.98 | 10.04 (5) |
| *Tagetes erecta* | 17.95de | 17.29–18.59 | 8.91 ± 0.64 | 8.24 (5) |
| *Allium cepa* | 18.52de | 17.94–19.09 | 11.30 ± 0.83 | 5.51 (5) |
| *Citrullus vulgaris* | 18.70de | 18.12–19.26 | 14.91 ± 1.15 | 6.52 (5) |
| *Cymbopogon tortilis* | 19.07de | 17.08–21.21 | 7.94 ± 1.19 | 20.49 (5) |
| *Capsicum annum* | 19.09de | 18.49–19.69 | 10.87 ± 0.80 | 8.16 (5) |
| *Alnus japonica* | 19.09de | 17.53–20.71 | 8.73 ± 1.09 | 14.41 (5) |
| *Ricinus communis* | 19.36de | 18.61–20.09 | 7.50 ± 0.50 | 8.66 (6) |
| *Gomphrena globosa* | 19.50de | 17.61–21.47 | 10.14 ± 1.61 | 23.04 (5) |
| *Ginkgo biloba* | 19.78de | 18.19–21.37 | 11.59 ± 1.65 | 18.63 (5) |
| *Momordica charantia* | 20.55e | 18.86–22.31 | 11.76 ± 1.84 | 20.45 (5) |
| *Plantago lanceolata* | 20.90e | 20.36–21.44 | 14.56 ± 1.15 | 6.25 (5) |
| *Piper Kadzura* | 21.35e | 19.87–22.91 | 13.38 ± 1.98 | 17.72 (5) |
| *Cleome spinosa* | 21.50de | 16.50–35.96 | 12.04 ± 4.16 | 103.07 (5) |
| *Arisaema takeshimense* | 21.51de | 17.56–27.97 | 9.16 ± 2.52 | 64.14 (5) |
| *Delphinium maackianum* | 21.69e | 20.15–23.28 | 9.16 ± 1.07 | 17.54 (6) |
| *Chrysanthemum indicum* | 21.87e | 19.05–25.61 | 10.72 ± 2.52 | 42.42 (5) |
| *Chrysanthemum coronarium* | 22.25de | 17.38–34.55 | 8.92 ± 2.84 | 80.31 (5) |
| *Artemisia annua* | 22.67e | 20.31–25.25 | 9.42 ± 1.64 | 37.51 (6) |
| *Datura metel* | 22.77e | 20.29–25.93 | 13.98 ± 3.27 | 41.16 (5) |
| *Citrus unshiu* | 22.86e | 21.39–24.36 | 12.84 ± 1.72 | 21.27 (6) |
| *Xylosma congestum* | 23.09e | 20.87–25.61 | 8.68 ± 1.39 | 30.74 (6) |
| *Chrysanthemum boreale* | 23.17e | 16.79–32.93 | 18.71 ± 6.76 | 94.56 (5) |
| *Hovenia dulcis* | 24.02e | 22.09–26.08 | 12.30 ± 2.03 | 31.59 (6) |
| *Nerium indicum* | 24.15e | 23.61–24.69 | 16.47 ± 1.28 | 4.80 (6) |
| *Humulus japonicus* | 24.48e | 22.91–26.28 | 27.58 ± 6.45 | 29.28 (5) |
| *Acer takeshimense* | 25.02e | 23.65–26.45 | 13.93 ± 1.88 | 18.30 (6) |
| *Rhododendron micranthum*[a] | - | - | - | - |
| *Chrysanthemum morifolium*[a] | - | - | - | - |

$LT_{50}$ values followed by different lower case letters are significantly different among treatments

[a]Larvae died faster than control, so $LT_{50}$ was not calculated

https://doi.org/10.1371/journal.pone.0198302.t003

*tabacum*+*A*. *sativum* was 140.1, indicating a synergistic effect of the mixture of these two extracts. However, in case of adult females, the $LT_{50}$ value was not significantly different between the mixture of *N*. *tabacum*+*A*. *sativum* and the mixture of *A*. *sativum*+*S*. *mukorossi* (Table 5). The mixture of *N*. *tabacum*+*A*. *sativum* showed 100% mortality within 144 h (Fig 4). The co-toxicity coefficient value of *N*. *tabacum*+*A*. *sativum* mixture was 107.5, indicating a synergistic effect of the mixture (Table 5), but, from the C. I. value, the mixture of *N*. *tabacum* +*A*. *sativum* was not significantly different from the single extract of *N*. *tabacum*. Here, we also found that adult males died faster than adult females in mixed extract treatment. From the above results, the mixture of *N*. *tabacum*+*A*. *sativum* would be the best choice for use against

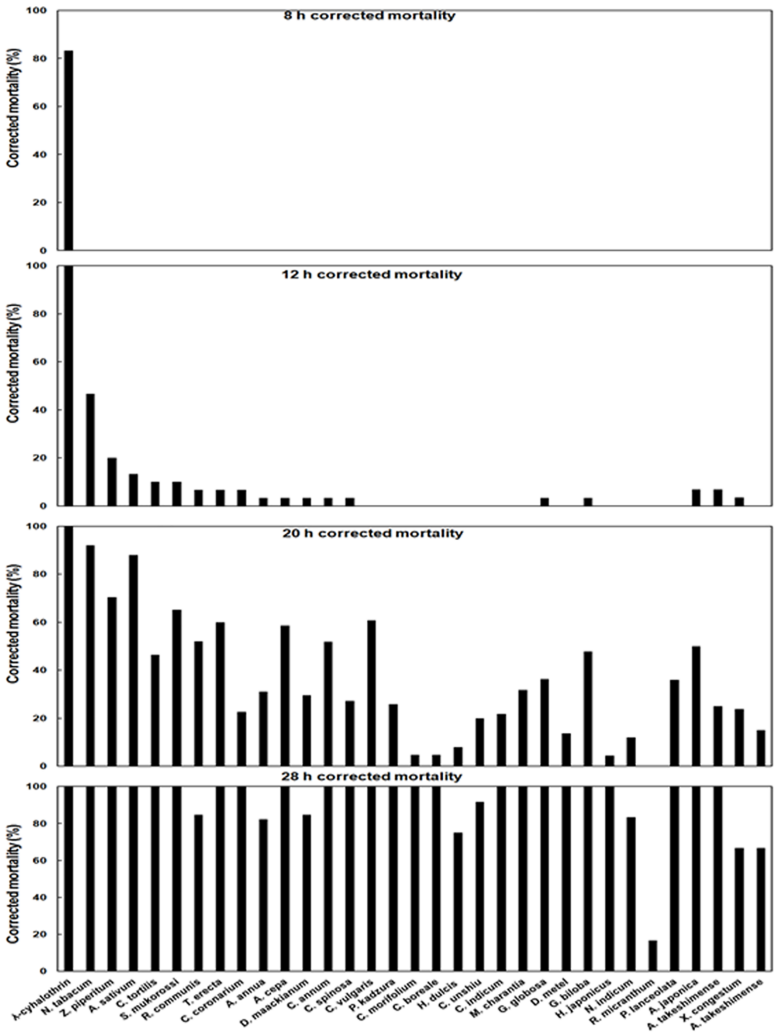


**Fig 1. Efficacy of different plant extracts against *Grapholita molesta* 1st instar larvae over time.**

https://doi.org/10.1371/journal.pone.0198302.g001

adult males, but the mixture of *N. tabacum*+*A. sativum* and *N. tabacum* by itself were both equally lethal to adult females.

## Greenhouse bioassay

In the greenhouse bioassay, infestation levels of twigs were significantly reduced when twigs were sprayed with either *N. tabacum* or *A. sativum* ($\chi^2 = 30.74$, $df = 5$, $P < 0.001$) compared to the negative control (Table 6). However, we found no significant differences among the plant extracts ($\chi^2 = 7.19$, $df = 3$, $P = 0.066$).

## Oviposition deterrence in the laboratory

From the above experiments we found that *N. tabacum*, *A. sativum*, and the mixture of *N. tabacum*+*A. sativum* provided the best control of adult *G. molesta*, so, these treatments were compared in an oviposition deterrence test in the laboratory. Mated females laid only 29 eggs on the leaves treated with *N. tabacum*, significantly fewer than all other plant extracts, and an 85% reduction compared to the methanol control ($\chi^2 = 236.50$, $df = 4$, $P < 0.001$) (Table 7).

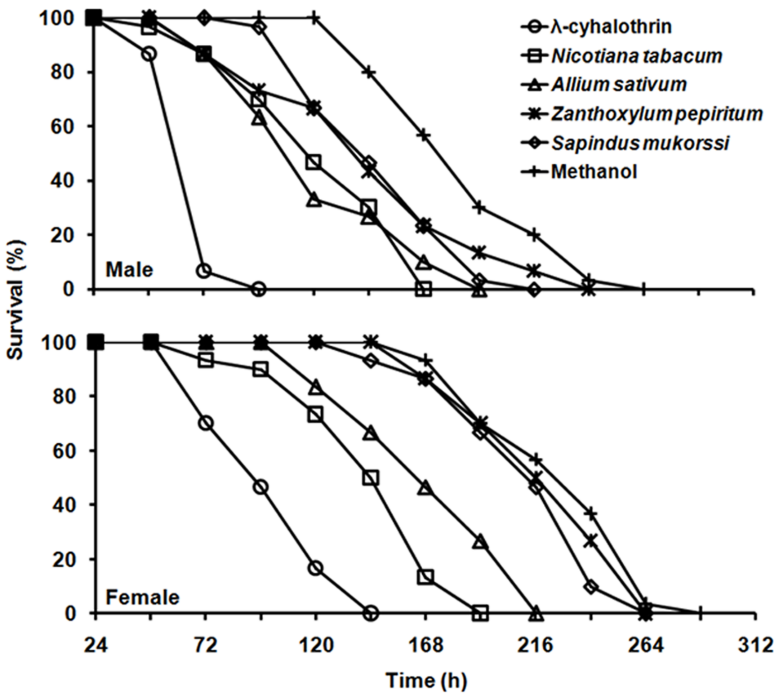


**Fig 2. Survivorship of adult male and female of *Grapholita molesta* after exposure to single applications of plant extracts.**

https://doi.org/10.1371/journal.pone.0198302.g002

We found *N. tabacum* to be very effective in reducing oviposition, at levels similar to those provided by λ-cyhalothrin, for up to three days (Fig 5).

## Oviposition deterrence the greenhouse

The number of eggs laid by adult mated females was significantly lower for all plant extracts compared to the negative control ($F = 9.82$, $df = 4, 9$, $P = 0.014$), and the percentage of leaves with eggs and the total number of eggs laid were reduced in the *N. tabacum* treatment by 71 and 90%, respectively, compared to the methanol control (Table 8).

**Table 4. Statistical comparison of tested methanolic plant extracts against adult *Grapholita molesta*.**

| Tested on | Treatment | $LT_{50}$ | 95% C.I. | Slope ± SE | $\chi^2$ (df) |
|---|---|---|---|---|---|
| Male | λ-cyhalothrin | 57.01a | 53.11–61.29 | 14.90 ± 2.53 | 0.01 (2) |
| | *Allium sativum* | 107.49b | 99.15–115.55 | 7.03 ± 0.83 | 3.08 (6) |
| | *Nicotiana tabacum* | 109.96bc | 101.17–119.41 | 6.43 ± 0.85 | 7.46 (5) |
| | *Zanthoxylum piperitum* | 126.35cd | 116.72–135.85 | 6.05 ± 0.63 | 5.85 (8) |
| | *Sapindus mukorossi* | 137.66de | 130.26–144.81 | 10.99 ± 1.33 | 2.96 (7) |
| | Methanol | 174.73f | 166.86–182.33 | 12.28 ± 1.39 | 3.17 (9) |
| Female | λ-cyhalothrin | 88.80a | 81.91–95.44 | 8.53 ± 1.20 | 3.77 (4) |
| | *Nicotiana tabacum* | 131.93b | 115.23–150.72 | 8.63 ± 1.65 | 14.39 (6) |
| | *Allium sativum* | 158.34bc | 150.23–166.77 | 10.44 ± 1.33 | 5.96 (7) |
| | *Sapindus mukorossi* | 201.46d | 193.66–209.54 | 13.87 ± 1.65 | 7.67 (9) |
| | *Zanthoxylum piperitum* | 209.58de | 201.74–217.78 | 14.49 ± 1.83 | 5.33 (9) |
| | Methanol | 215.49ef | 207.77–223.23 | 15.15 ± 1.76 | 6.66 (10) |

$LT_{50}$ values followed by different letters are significantly different among treatment.

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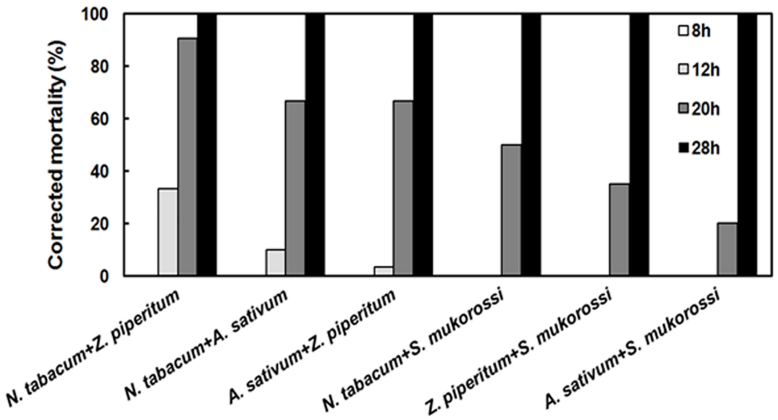


**Fig 3. Corrected mortality (%) of combinations of plant extracts against first instar larvae of *Grapholita molesta*.**

https://doi.org/10.1371/journal.pone.0198302.g003

## HPLC analysis

Nicotine the major compound of *N. tabacum* appeared 56.3% at RT 2.42 min with two unidentified minor compounds at RT 2.83 min (27.01%) and 3.77 min (10.13%) (Fig 6). From *A. sativum*, the major compound allicin appeared 100% at RT 3.19 min (Fig 7).

**Table 5. Statistical comparison of tested methanolic plant extracts (mixture) against *Grapholita molesta*.**

| Tested on | Treatment | $LT_{50}$ | 95% C.I. | Slope ± SE | $\chi^2$ (df) | Co-toxicity coefficient |
|---|---|---|---|---|---|---|
| Larva[a], first instar | λ-cyhalothrin | 5.32a | 4.92–5.72 | 6.21 ± 0.58 | 2.35 | - |
| | *N. tabacum+Z. piperitum* | 14.27b | 12.78–15.65 | 9.03 ± 1.17 | 11.32(4) | 90.54 |
| | *N. tabacum+A. sativum* | 18.20c | 16.52–19.90 | 8.31 ± 1.08 | 16.26(5) | 70.99 |
| | *A. sativum+Z. piperitum* | 18.04c | 17.47–18.60 | 11.40 ± 0.84 | 2.51(5) | 86.31 |
| | *N. tabacum+S. mukorossi* | 18.99cd | 17.83–20.10 | 12.44 ± 1.44 | 11.38(5) | 68.04 |
| | *A. sativum+S. mukorossi* | 21.80cde | 19.81–24.05 | 12.95 ± 2.45 | 28.37(5) | 71.42 |
| | *Z. piperitum+S. mukorossi* | 21.65cdef | 18.56–25.79 | 9.65 ± 2.34 | 43.92(5) | 74.32 |
| Adult, male | λ-cyhalothrin | 54.87a | 48.10–60.78 | 7.97 ± 1.54 | 1.67 (2) | - |
| | *N. tabacum+A. sativum* | 76.70b | 68.37–84.76 | 6.19 ± 0.91 | 2.87 (4) | 140.14 |
| | *A. sativum+S. mukorossi* | 94.48c | 86.63–101.98 | 8.35 ± 1.17 | 3.04 (5) | 113.77 |
| | *N. tabacum+Z. piperitum* | 100.13cd | 91.88–108.11 | 8.06 ± 1.14 | 1.84 (5) | 109.82 |
| | *N. tabacum+S. mukorossi* | 122.87e | 115.50–129.94 | 12.15 ± 1.72 | 0.74 (6) | 89.49 |
| | *Z. piperitum+A. sativum* | 123.65e | 114.92–132.41 | 8.58 ± 1.15 | 2.86 (6) | 86.93 |
| | *Z. piperitum+S. mukorossi* | 135.43ef | 127.90–142.82 | 12.69 ± 1.82 | 1.28 (6) | 93.30 |
| | Methanol | 170.30g | 161.87–178.65 | 12.45 ± 1.66 | 6.74 (8) | - |
| Adult, female | λ-cyhalothrin | 86.03a | 78.37–93.50 | 8.01 ± 1.18 | 1.79 (4) | - |
| | *N. tabacum+A. sativum* | 122.69b | 112.66–132.71 | 6.51 ± 0.80 | 6.51 (7) | 107.53 |
| | *A. sativum+S. mukorossi* | 140.15bc | 131.65–148.40 | 10.36 ± 1.36 | 1.71 (7) | 112.98 |
| | *Z. piperitum+A. sativum* | 156.65cd | 147.49–165.68 | 9.85 ± 1.21 | 3.55 (8) | 101.08 |
| | *N. tabacum+Z. piperitum* | 175.50e | 166.48–184.81 | 11.21 ± 1.46 | 4.49 (8) | 75.17 |
| | *N. tabacum+S. mukorossi* | 187.83ef | 178.40–197.59 | 10.81 ± 1.31 | 9.03 (9) | 70.24 |
| | *Z. piperitum+S. mukorossi* | 231.07h | 223.06–239.38 | 18.18 ± 2.40 | 7.66 (10) | 90.70 |
| | Methanol | 200.61fg | 191.94–209.00 | 13.99 ± 1.72 | 2.74 (10) | - |

$LT_{50}$ values followed by different letters are significantly different among treatment

[a]The $LT_{50}$ value was calculated using corrected mortality

https://doi.org/10.1371/journal.pone.0198302.t005

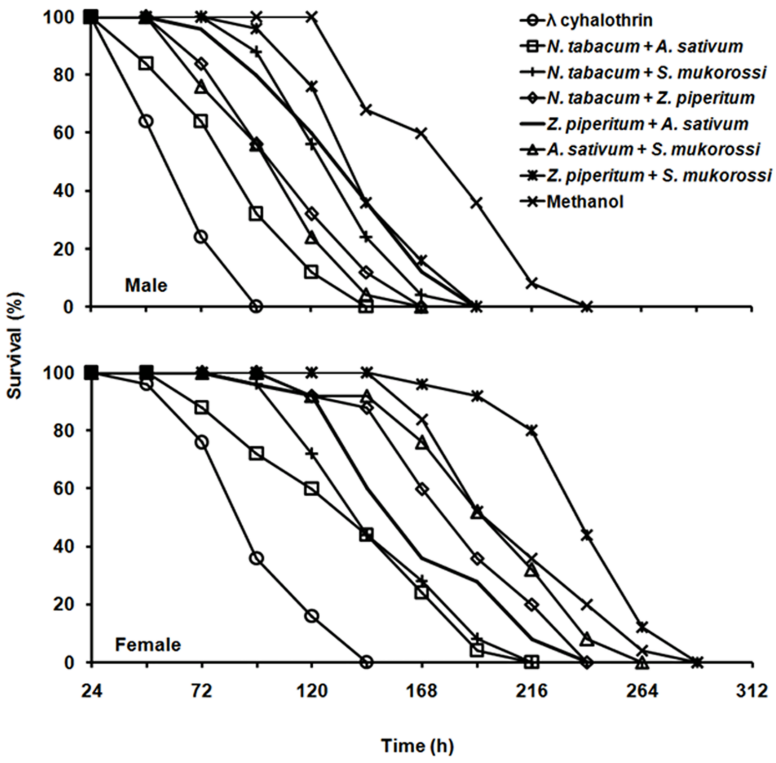


**Fig 4. Survivorship of adult male and female of *Grapholita molesta* on mixed application of plant extracts.**

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## Discussion

The synthetic pesticide λ-cyhalothrin was more toxic than any of plant extracts to first instar larvae. Based on the comparison of plant extract $LT_{50}$ values to that of λ-cyhalothrin, we selected *N. tabacum*, *A. sativum*, *Z. piperitum*, and *S. mukorossi* as the most effective botanical extracts for control of first instar larvae of *G. molesta*. Although the highest mortality was observed in larval stage of *G. molesta* from *N. tabacum* treatment, for both adult males and females *N. tabacum* and *A. sativum* were equally effective in a subsequent assay. *Nicotiana tabacum* has several modes of action. It can be a nerve poison [27, 28], stomach poison, or repellent [29]. Baskaran and Narayanasamy [29] found *N. tabacum* to be effective against aphids, thrips, psyllids, tingids, beetles, sawflies, and lepidopterans. Evaluation of *N. tabacum* against *G. molesta* has been made here for the first time. In addition, *N. tabacum* is easy to apply in the field. Amoabeng et al. [16] ground *N. tabacum* leaves in tap water containing 0.1%

**Table 6. Efficacy evaluation of plant extracts on infestation rate of peach twigs in greenhouse.**

| Treatment | Hatchability (%) | Infestation rate |
|---|---|---|
| λ-cyhalothrin | 88.0 | 0.09 (2/22)d |
| *Nicotiana tabacum* | 88.0 | 0.27 (6/22)cd |
| *Allium Sativum* | 84.0 | 0.38 (8/21)bdc |
| *Zanthoxylum piperitum* | 88.0 | 0.45 (10/22)abcd |
| *Sapindus mukorssi* | 84.0 | 0.67 (14/21)abc |
| Control | 88.0 | 0.82 (18/22)a |

Means within a column with different letters differ significantly ($P < 0.05$)

https://doi.org/10.1371/journal.pone.0198302.t006

**Table 7. Deterrent effect of plant extract on oviposition of *G. molesta* in laboratory.**

| Treatment | Total no. of eggs produced | % eggs on wall | % eggs on leaves |
|---|---|---|---|
| λ-cyhalothrin | 267 | 97.75a | 2.25a |
| *Nicotiana tabacum* | 312 | 90.71b | 9.29b |
| *N. tabacum+A. sativum* | 319 | 67.71c | 32.29c |
| *Allium sativum* | 377 | 64.99c | 35.01c |
| methanol | 396 | 51.77d | 48.23d |

Means within a column with different letters differ significantly ($P < 0.05$)

https://doi.org/10.1371/journal.pone.0198302.t007

Sunlight® detergent solution and sieved them through fine linen for immediate application to a cabbage field. This preparation resulted in 93.0% reduction of *Plutella xylostella* larvae, while λ-cyhalothrin reduced the same population by only 51.0%. The best efficacy was recorded with the extract of *N. tabacum* against *Cydia molesta* Busch. (98.3%) and *Anarsia lineatella* Zell. (99.0%) [30]. Vandenborre et al. [27] found that a jasmonate-inducible lectin named NICTABA present in tobacco leaf is responsible for the larval mortality of lepidopteran insects. Nevertheless, a major active compound of *N. tabacum* was nicotine, which mimics acetylcholine and activates the nicotinic acetylcholine receptor causing an influx of sodium ions to flood the receptor [28]. Methanolic extracts of *A. sativum* have also caused mortality of 81.0% against *Spodoptera litura* [31]. A constituent of the *A. sativum* extract, alliin (derived from the amino acid cysteine) is converted by an enzyme to allicin, which is believed to act as an antifeedant, repellent, and insecticide [32].

We did not find any synergistic effects of *N. tabacum* and *Z. piperitum* on first instar larvae of *G. molesta*. However, the mixture of *N. tabacum+A. sativum* showed synergistic effects on adult males. The reason for this difference in the effectiveness of the mixture of *N. tabacum+A.*

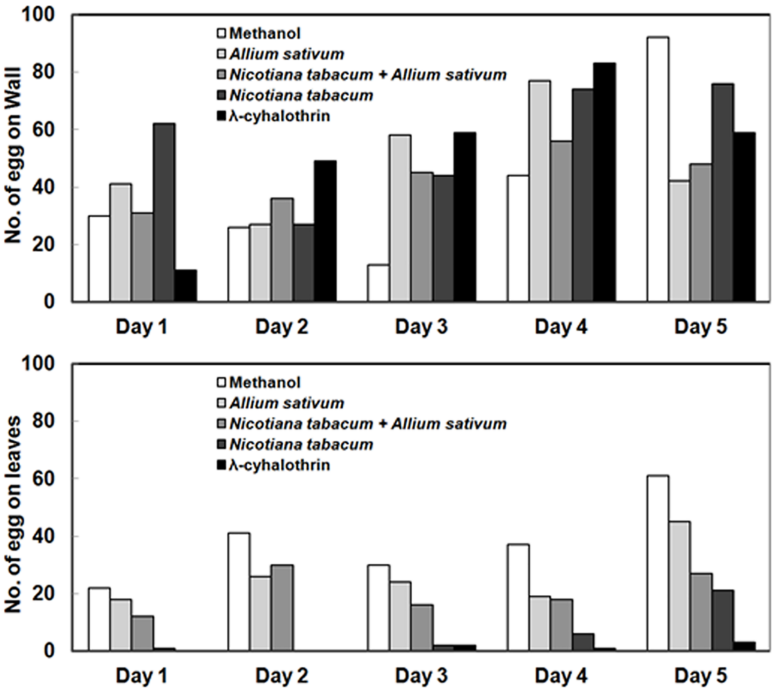


**Fig 5. Daily egg laying on cage walls and leaves up to five days.**

https://doi.org/10.1371/journal.pone.0198302.g005

**Table 8. Deterrent effect of plant extract on oviposition of *G. molesta* on greenhouse.**

| Treatment | No. of leaves/twig | Percent of twigs of which leaves with egg | Percent of leaves with egg | Total no. of eggs reproduced |
|---|---|---|---|---|
| λ-cyhalothtrin | 9.36 (103/11) | 0.00 (0/11)a | 0.00 (0/103)a | 0c |
| *Nicotiana tabacum* | 8.56 (94/11) | 36.36 (4/11)ab | 8.51 (8/94)b | 18b |
| *Allium sativum* | 6.79 (95/14) | 57.14 (8/14)b | 15.79 (15/95)b | 28b |
| *N. tabacum+A. sativum* | 9.00 (117/13) | 69.23 (9/13)b | 19.67 (23/117)bc | 42b |
| methanol | 7.15 (93/13) | 46.15 (6/13)b | 29.03 (27/93)c | 184a |

Means within a column with different letters differ significantly ($P < 0.05$)

https://doi.org/10.1371/journal.pone.0198302.t008

*sativum* between larvae and adults is unknown, but might be caused by differences in physiological structure. Similarly, Derbalah [33], who found that an extract of *Bauhinia purpurea* L. showed 83 and 80% mortality on adult and pupal stages of *Trogoderma granarium* Everts, respectively, but only 33.0% mortality on the larval stage. Interestingly, extracts of *Caesalpinia gilliesii* (Hook.) showed lower mortality on adult and pupal stages (43.0 and 43.0%, respectively), than on larvae (80%).

We found no synergistic effect of *N. tabacum* and *Z. piperitum* on the first instar larvae of *G. molesta*, and similarly Noosidum et al. [34] found no synergistic effect of the mixture of *Litsea salicifolia* Roxb. (0.1%) and *Melaleuca leucadendron* L. (0.3%) against adult females of *Aedes aegypti* (L.). However, the synergistic effects of mixtures of plant extracts have been reported in other studies. Alim et al. [35] found that a mixture of neem plus crown flower at a 1:1 ratio showed synergistic effects on *Aleurodicus dispersus* adults. Zibaee and Khorram [36] also found that essential oils of *Eucalyptus globulus* Labill. and *Rosmarinus officinalis* L. showed synergistic effects on *Blattella germanica* L.

*Nicotiana tabacum* extract was effective in deterring oviposition in both laboratory and greenhouse assays, which suggests it would be effective at reducing *G. molesta* populations in the field. Similarly, Amoabeng et al. [16] found that *N. tabacum* extract reduced 93.0% of a *Plutella xylostella* population in a cabbage field. In other work in Uganda, a crude extract of *N. tabacum* showed similar effectiveness to the synthetic insecticides against a bruchid beetle (*Callosobruchus* sp.) [37]. Nevertheless, plant extracts can be harmful to other beneficials: *N. tabacum* found to be harmful on *Coccinella magnifica* Redtenbacher and *Episyrphus balteatus* De Geer compared to tap water but less harmful than synthetic insecticides [16].

In conclusion, among the 32 tested plant extracts, *N. tabacum* extract showed highest toxicity against the first instar and adult of *G. molesta*, and oviposition was greatly reduced after the spray

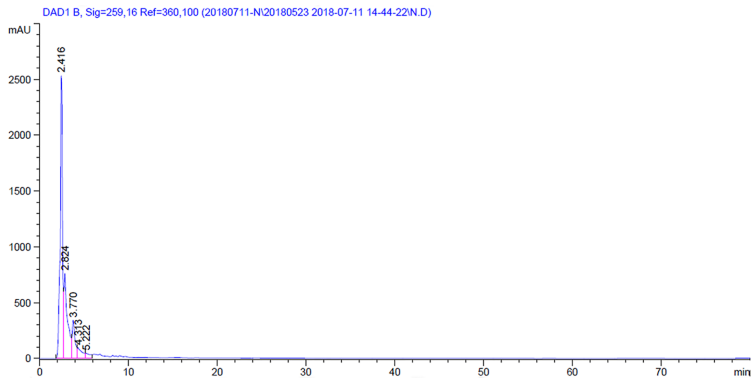


**Fig 6. HPLC of methanol extract of *Nicotiana tabacum*.**

https://doi.org/10.1371/journal.pone.0198302.g006

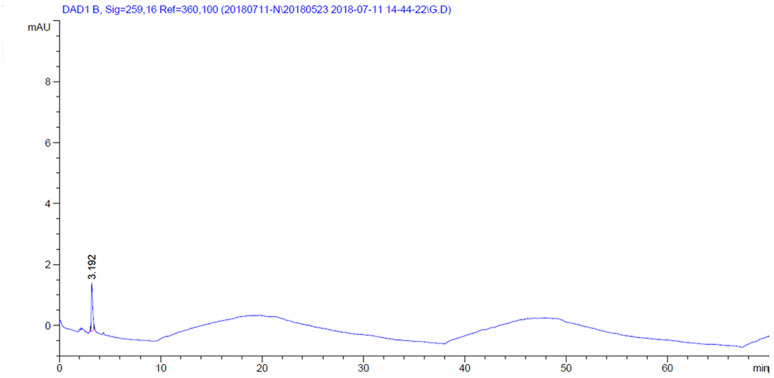


**Fig 7. HPLC of methanol extract of *Allium sativum*.**

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in both laboratory and greenhouse. Nevertheless, formulation should be improved as methanolic extracts in this study is not appropriate for organic farming. Based on these results, we are suggesting that the extract of *N. tabacum* can be a good botanical insecticide against *G. molesta*.

## Supporting information

**S1 File. Test of single plant extract on larva, test of single plant extract on adult, test of combination of extracts on larva, test of combination of extracts on adult, Greenhouse evaluation of plant extracts, Oviposition deterrency in laboratory, Oviposition deterrency in greenhouse.**
(XLSX)

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