Plant oil mixtures as a novel botanical pesticide to control gregarious locusts

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Abstract

For thousands of years, large locust swarms have caused severe problems in agriculture. People fight against current outbreaks by using chemical pesticides or an insect fungus known as Green Muscle™. While chemical pesticides may be harmful for humans and non-target species, the sporulation of the fungus takes a long period of time and requires conditions of high humidity that are not always found in the field. In this study, we tested the toxicity of a linseed oil/bicarbonate emulsion against gregarious desert locusts and screened for plant essential oils that enhance its toxicity. Finally, we combined three essential oils to develop a novel formulation that is effective against the desert and migratory locust species after single spray treatment. Within 24 h, this formulation caused a mean mortality rate of 80% and 100% of desert and migratory locusts, respectively. Its toxicity is based on a synergistic effect resulting from the combination of caraway, orange peel and winter-green oils. In addition, we tested this botanical pesticide on two beetle species regarded either as alternative or beneficial non-target species. The first species, mealworm beetles, did not suffer from the spray treatment and behaved normally after 8 days. In contrast, 67.7% of ladybird adults died in the same time span. Interestingly, the growth of wheat seedlings was almost unaffected by spraying this botanical pesticide. These results suggest this botanical pesticide can be used as a strong agent against desert and migratory locusts, but needs to be used with care to minimize unwanted side effects on the ecosystem.

Keywords Locust swarm · Linseed oil · Essential oils · Mealworm beetles · Ladybird beetles · Pest management · Botanical pesticide

Key message

- In many countries, locust outbreaks still constitute a big problem for farmers. Current pest management is potentially harmful for humans.
- We developed a novel botanical pesticide against locusts that consisted of natural plant oils.
- We identified orange peel, caraway and wintergreen oils to be effective against locusts when added to a linseed oil/bicarbonate emulsion.
- Our novel botanical pesticide had a synergistic toxic effect.
- This formulation is highly effective against two gregarious locust species and is considered harmless for humans.

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Introduction

Locusts constitute a major problem for agricultural crops (Wright 1986; Brader et al. 2006; Millist and Abdalla 2011; Latchininsky 2013; Zhang and Hunter 2017). They can transform reversibly between two extreme phases, solitary and gregarious. The locusts differ in both of these phases in terms of their morphology, physiology and behavior (Steedman 1988; Uvarov 1966). The distribution of gregarious locusts extends from Africa through the Middle East to Asia,

South and Central America, Australia and Southern Europe. When these species transform into the gregarious phase, they form locust swarms that can devour entire fields and cause extensive crop damage over a very short period of time. A small swarm of locusts contains thousands of individuals that spread over several hundred square meters, but large swarms contain up to 80 million individuals per km² and can infest more than 1000 km². Since a swarm can cover a distance of 100 km per day (Steedman 1988), farmers regard gregarious locusts as one of the most destructive plagues on earth.

Before 1980, persistent organochlorine compounds like dieldrin were used extensively to combat locust swarms (Steedman 1988). Because organochlorine compounds have been banned, locust control is currently mainly based on organophosphorus pesticides. The reduced persistence of these compounds makes them less effective than dieldrin (Arthurs 2008). From 2003–2005, authorities applied thirteen million liters of chemical pesticides to thirteen million hectares of land to control desert locust outbreaks (Lecoq 2010). This intensive use of chemical pesticides is probably underestimated and has led to severe environmental problems, which have had harmful side effects on the overall ecosystem (WHO 1990, 2012; Alavanja 2009; Alavanja and Bonner 2012; Köhler and Triebskorn 2013; Carvalho 2017). Therefore, there is an interest in developing potentially safe alternatives or additional control technologies (Nicolopoulou-Stamati et al. 2016). Natural enemies cannot be used because of the rapid development and migration of locusts (Steedman 1988). Over the past 20 years, the Food and Agriculture Organization of the United Nations (FAO) has been working with several countries to develop a bio-pesticide that is based on the fungus *Metarhizium acridum*, which infests locusts and grasshoppers (Lecoq 2010). The commercial name of this bio-pesticide is Green Muscle™. In 2009, the desert Locust Control Committee located in Rome recommended that properly trained professional teams use Green Muscle™ operationally for the preventive control of the desert locust when the temperature is suitable (FAO 2009). Because Green Muscle™ has several shortcomings, such its long incubation period, scientists urgently need to carry out more studies to develop effective agents that control locusts and do not have severe side effects on the ecosystem.

In recent years, plant oils have received more and more attention as alternative, potentially valuable compounds that can be used against specific pests and some important plant pathogens (Isman 2004). Several studies have confirmed that plant oils could be used as natural pesticides to control agricultural pests (e.g., Aly et al. 2012; Tak 2015; Tripathi et al. 2009) and plant diseases (e.g., Arshad et al. 2014; Sturchio et al. 2014; EL Rasheed and EL Rasheed 2017; Elshafie and Camele 2017). Most natural plant oils are suitable for organic agriculture because they are non-residual, non-toxic to humans and vertebrate animals, easy to prepare and apply, cheap, effective

and natural (Elshafie and Camele 2017). Plant oils are available as vegetable oils and essential oils. The first type is produced by expressing the oils from the seeds and parts of the plant (e.g., sunflower oil, olive oil, linseed oil). The second type is extracted from the plants by means of distillation (e.g., orange oil, peppermint oil, garlic oil, lavender oil). Essential oils are volatile compounds and must be diluted in carrier oils before their use. For centuries, plant oils or extracts have been used in alternative medicine, aromatherapy, as food flavorings, perfumes, preservatives and biological agents (Tak 2015). Recent studies have suggested that essential oils are effective against locusts and grasshoppers. For example, Sharaby et al. (2012) suggested that the essential oils of garlic, eucalyptus and mint could be used in the integrated pest management (IPM) against the grasshopper *Heteracris littoralis*. Abdellah et al. (2013) tested the effect of the crude leaf essential oil obtained from *Peganum harmala* L. (wild rue) on larvae and adult individuals of the desert locust. They reported that the desert locusts displayed toxic effects, imbalance problems and convulsive movements. Halawa and Hustert (2014) found that lovage essential oil and its main components, limonene, impaired the ventilation of locusts at low concentrations. Lahsen et al. (2015) found that rosemary essential oil displayed a remarkably toxic effect against the moroccan locust (*Dociostaurus maroccanus*). In all of these studies, essential oils were found to affect locusts in different ways, but oils have not been tested on non-target species, such as beetles that are known to play important roles in the ecosystem.

In this study, we developed a novel mixture of plant oils that has highly toxic effects on two target species of gregarious locusts: the desert locust *Schistocerca gregaria* and the migratory locust *Locusta migratoria*. The intension was to coat the locusts with a thin layer of an oil that is difficult for the insect to remove. We used linseed oil, a drying oil that becomes viscous over time. In order to speed up the drying process, we mixed it with a saturated solution of sodium bicarbonate, which may have an additional benefit in that it is used for the control of fungal plant diseases (Horst et al. 1992; Kuepper et al. 2001). In order to increase the toxic effect of the linseed emulsion, we added three essential oils that were found to be toxic for desert locusts in a screening study. The toxic effect of the combination of effective essential oils sprayed at a low concentration on two locust species was studied 24 h after single spray treatment. To test the toxic effect of this botanical pesticide on a beneficial non-target species and an alternative non-target species, we also sprayed adult ladybirds and their larvae, as well as adult mealworm beetles. We selected ladybird beetles as a non-target species because of its important role in the ecosystem (e.g., control of plant lice) and also treated mealworm beetles to determine whether the pesticide has a possibly harmful effect on another small beetle species. To study the effect of this formulation on the growth of grass, we sprayed it on wheat seedlings and exposed them to sunlight.

Materials and methods

Insects

Desert locusts (*Schistocerca gregaria*) and migratory locusts (*Locusta migratoria*) were purchased in the gregarious phase from a breeding stock of the Buchner company in Austria. Locusts were maintained in a crowded colony at the Institute of Zoology in Graz. About 100 locusts were kept in a glass terrarium with dimensions of 60×30×30 cm. Individuals used in this study were taken from crowded colonies about 2 weeks after their last molt. The light/dark cycle was 12:12 h, and the average temperature in the terrarium was 26 ± 2 °C (mean $\pm$ SD) at night and 33 ± 2 °C (mean $\pm$ SD) during the day. The relative humidity was 45–60%. The locusts' diet consisted of organic wheat seedlings and organic wheat bran (dm-Drogerie markt, Karlsruhe, Germany), both in the breeding stock and in the crowded colony.

Adults and larvae of the mealybug ladybird (*Cryptolaemus montrouzieri*) were purchased from a breeding stock of the Biohelp company (Vienna, Austria). Ladybird adults were kept in plastic boxes with dimensions of 20×12×14 cm, each containing 30 individuals. About 100 ladybird larvae (final larval instar) were kept in a plastic box with dimensions of 20×12×14 cm, covered with transparent fabric, which contained a small piece of paper towel (12×12 cm). The light/dark cycle was 12:12 h, the temperature was 22 ± 2 °C (mean $\pm$ SD), and the relative humidity was $40 \pm 16\%$ (mean $\pm$ SD). The diet for ladybird adults and larvae consisted of sweetened water gel (2 ml of 50% (g/V) glucose solution added to 10 g water gel (Trixie Heimtierbedarf, Tarp, Germany)).

Adult mealworm beetles (*Tenebrio molitor*) were taken from the laboratory breeding stock maintained at the Institute of Biology in Graz. Beetles were exposed to a light/dark cycle of 12:12 h and maintained at an ambient temperature of 22 °C and 50% relative humidity. Their diet consisted of dry bread, apples, fish food and oatmeal (SPAR Österreich Warenhandels AG, Salzburg, Austria).

Feeding plants

Seeds of organic wheat (dm-Drogerie markt, Karlsruhe, Germany) were planted in plastic pots that had been filled halfway with perlite (Plant!t, HydroGarden Wholesale Supplies Ltd, Binley, Coventry, CV3 2NT, UK) and covered with soil (Organic soil from EuFlor Inc., Munich, Germany). Pots were watered daily, and plants were exposed to a light/dark schedule of 12: 12 h. After 1 week, the wheat seedlings were placed in the terrarium to feed the locusts.

Oil emulsions and suppliers

In this study, we used linseed oil as insecticidal agent to impair the motion of locusts by means of an oil that becomes viscous over time. In order to accelerate the drying process of this oil, the linseed oil was mixed with a saturated solution of sodium bicarbonate.

The linseed/bicarbonate emulsion consisted of:

Cold-pressed organic linseed oil (*Linum usitatissimum*), (Natur-Pur brand, Spar Österreich) and saturated sodium bicarbonate solution (IUPAC name: sodium hydrogen carbonate NaHCO_3 , commercial name: baking soda), purchased from Dr. Oetker, Villach, Austria.

We assessed the hardening process of the linseed/bicarbonate emulsion (56:44% (v/v)) in a petri dish for 1 day (see Fig. 1). Linseed oil was stored in a dark bottle at 4 °C.

To increase the harmful effect caused by the hardening of the linseed oil emulsion, the following organic essential oils were added individually and tested for their insecticidal effects:

Peppermint oil (*Mentha piperita*), orange peel oil (*Citrus aurantium dulcis*), ginger oil (*Zingiber officinale*) and eucalyptus oil (*Eucalyptus globulus*). These oils were purchased from Primavera (Oy-Mittelberg, Germany).

Basil oil (*Ocimum basilicum*) and wintergreen oil (*Gaultheria procumbens*), both purchased from Naissance (Neath, UK).

Garlic oil (*Alium sativum*) purchased from Dragonspice Naturwaren (Reutlingen, Germany).

Caraway oil (*Carum carvi*) purchased from Bombastus-Werke (Freital, Germany).

Clove oil (*Eugenia carophyllus*) purchased from Green-Made (V03 Trading GmbH, Willich, Germany).

Birch oil (*Betula lenta*) purchased from Laboratoire Centiflor (Entrehiaux, France).

All essential oils were 100% pure and were stored at room temperature in a dark location. The selection of these essential oils was based on the findings of previous scientific studies in which some of these essential oils (or oils with similar effective components) were investigated for their effects against various insects such as termites, locusts, moths and beetles (Aly et al. 2012; Halawa and Hustert 2014; Tak et al. 2016, 2017; Tripathi et al. 2009).

Preparation of the linseed/bicarbonate emulsion

The saturated sodium bicarbonate solution was prepared by dissolving 1 g NaHCO_3 powder in 10 ml distilled water

Fig. 1 Hardening of the linseed-bicarbonate emulsion. **a** Image of the emulsion after mixing linseed oil and bicarbonate solution. **b** Image of the viscous film formed after 24 h

(10%). The supernatant was then added to the linseed oil using a 50 ml plastic centrifuge tube. Essential oils were added to this emulsion using an automatic calibrated pipette (Eppendorf Inc.). All oil emulsions were mixed with a vortex device (VF2, Ika labortechnik, Janke & Kunkel, Germany) before spray treatment.

Screening for effective essential oils

To screen for essential oils that are effective against desert locusts (*S. gregaria*), we sprayed 4.9 ml of different oil mixtures (equal to five pump sprays) into each box containing ten individuals. Since the preliminary experiment had demonstrated a higher robustness of desert locusts against linseed/bicarbonate treatment as compared with migratory locusts, we selected the former for this screening study. The number of living and dead individuals was counted after 24 h by gently shaking the insect boxes. Individuals that were unable to move and breathe normally (absent telescopic abdominal movement) were regarded as dead in this study, because ‘frozen’ individuals did not recover from this state during the observation period of

40 h. The effects caused by essential oils were compared with three control situations.

Controls and treatments used in the screening experiment:

Control 1: no treatment

Control 2: spraying pure linseed oil

Control 3: spraying saturated sodium bicarbonate solution (10%)

Treatments:

Linseed/bicarbonate emulsion: 10 ml linseed oil (55.6%) + 8 ml aqueous 10% sodium bicarbonate solution (44.4%)

1 ml pure essential oil (garlic, caraway, clove, peppermint, orange, ginger, birch, eucalyptus, or basil), each was added to 18 ml linseed/bicarbonate emulsion (resulting proportions: 5.3% essential oil, 52.6% linseed oil and 42.1% sodium bicarbonate solution).

Adult desert locusts (mature: about 2 weeks after last molt) were placed in plastic boxes with dimensions of 20 × 12 × 14 cm. Individuals used in experiments appeared

healthy and were able to jump. The boxes were covered with transparent fabric. Each box contained ten locusts (half females and half males), a small piece of paper towel (one layer of paper tissue of 12 × 12 cm) and some wheat grass (about 35 stalks). After shaking the oil emulsion, 4.9 ml was sprayed on the insects and their food using a pump sprayer (hand spray device; Kläger Plastik, Neusäss, Germany; nozzle diameter = 0.6 mm). The food was also sprayed to screen for the oral toxicity and contact-dependent toxic effects caused by essential oils. The insect boxes were turned by 90° to spray the locusts from top (distance between the sprayer and locusts ≈ 20 cm) and keep the aerosol inside the box. The same amount of oil emulsion that was applied to insects was also sprayed on a weighing dish to quantify the applied volume. The paper towel in the boxes soaked up the remaining oil emulsion.

Effect of the combination of essential oils

Essential oils that contributed to a high mortality rate of adult desert locusts (orange peel, birch oil and caraway oil) were combined in different concentrations to test different botanical pesticide formulations as agents against locusts. Garlic oil has a strong smell and was not used in these formulations. We also replaced birch oil by wintergreen oil, since it is more readily available and has the same active ingredient (methyl salicylate). Finally, two slightly different formulations caused a high mortality in desert and migratory locusts (data not shown). The botanical pesticide formulation used in this study (see description below) was found to be the most effective. In order to reveal a possible synergistic effect caused by the combination of three essential oils, we performed a ‘synergism experiment.’ In this experiment, essential oils were sprayed at low concentrations either individually or in combination (termed botanical pesticide treatment) on desert and migratory locusts. We specifically compared the mortality rate caused by orange peel, caraway and wintergreen oils with a botanical pesticide formulation that contains all of these oils. The treatment of locusts in the synergism experiment was similar to the treatment used during the screening experiment with the exception that fresh grass was provided after the spray treatment (4.9 ml was sprayed in control 2 and each treatment). This allowed us to discriminate between a toxic effect caused by physical contact with the oil emulsion and oral toxicity. The following controls and treatment were tested:

Control 1: no treatment

Control 2: Linseed/bicarbonate emulsion: 10 ml linseed oil (55.6%) + 8 ml aqueous 10% sodium bicarbonate solution (44.4%)

Caraway treatment: Linseed/bicarbonate emulsion consisting of 10 ml linseed oil (52.6%) and 8.5 ml aqueous 10% sodium bicarbonate solution (44.7%) + 0.5 ml caraway oil (2.6%)

Orange peel oil treatment: Linseed/bicarbonate emulsion consisting of 10 ml linseed oil (52.6%) + 8.75 ml aqueous 10% sodium bicarbonate solution (46.1%) + 0.25 ml orange peel oil (1.3%)

Wintergreen treatment: Linseed/bicarbonate emulsion consisting of 10 ml linseed oil (52.6%) + 8.75 ml aqueous 10% sodium bicarbonate solution (46.1%) + 0.25 ml wintergreen oil (1.3%)

Botanical pesticide treatment: 18 ml linseed/bicarbonate emulsion (10 ml linseed oil (52.6%) and 8 ml aqueous 10% sodium bicarbonate solution (42.1%)) + 0.25 ml orange peel oil (1.3%) + 0.5 ml caraway oil (2.6%) + 0.25 ml wintergreen oil (1.3%).

Each treatment was repeated five times, and the average mortality rates were calculated.

LC₅₀ test of the botanical pesticide

The lethal concentration 50% (LC₅₀) of our botanical pesticide formulation was assessed within 40 h to reveal whether it had been applied at a concentration that was effective to control desert locusts, which had been found to have a lower mortality rate as compared to migratory locusts. For this purpose, we diluted the botanical pesticide (same composition as in the synergy experiment) with distilled water to create different concentrations and sprayed it on adult desert locusts (*S. gregaria*). A similar treatment procedure was used as in the screening test, but fresh grass was added after the spray treatment. Individuals were counted either as dead or alive when they did not move and breathe after gently shaking the box. LC₅₀ experiments were repeated three times, and the average mortality rate was calculated. The dilutions of the botanical pesticide for the LC₅₀ experiment were:

5 ml botanical pesticide (25%) + 15 ml distilled water (75%)

10 ml botanical pesticide (50%) + 10 ml distilled water (50%)

15 ml botanical pesticide (75%) + 5 ml distilled water (25%)

18 ml botanical pesticide (90%) + 15 ml distilled water (10%)

20 ml botanical pesticide (100%)

The LC₅₀ was calculated after performing a linear regression on the averaged mortality rates obtained at different concentrations.

Botanical pesticide treatment of ladybirds

Ladybirds play an important role in many ecosystems and were considered to be a beneficial non-target species in this study. To determine whether our botanical pesticide formulation (see above) could possibly harm ladybirds, we sprayed adults and larvae of the mealybug ladybird species *C. montouzieri* in an additional experiment. Thirty ladybird adults and 15 ladybird larvae, all retrieved from the purchased colony, were put in separate plastic boxes with dimensions of 20 × 12 × 14 cm. The boxes were covered with transparent fabric. Each box contained a small piece of paper towel (12 × 12 cm) to soak up the remaining oil. Due to the very small size of ladybird species, we reduced the amount of the sprayed volume to only 2 ml of the botanical pesticide formulation to prevent the submersion of individuals. The oil emulsion was sprayed from above in each box (equal to two pump sprays). This experiment was repeated four times with adults. In each box, a small petri dish containing 10 g of water gel (Trixie Heimtierbedarf, Tarp, Germany) with 2 ml glucose solution (50% (g/V)) was provided as food, initially after the spraying treatment and then on a daily basis.

Botanical pesticide treatment of mealworm beetles

To determine whether our botanical pesticide formulation (see above) had a possibly harmful effect on an alternative non-target species, adult mealworm beetles (*T. molitor*) were sprayed in an additional experiment. For this purpose, 16 mealworm individuals were caged in plastic boxes with dimensions of 9 × 9 × 6 cm that had pores on two sides to allow for ventilation. Individuals used in this experiment appeared to be healthy. All boxes contained a small piece of an apple, 3.5 g of oat flakes and 0.5 g of fish food (ASTRA Aquaria GmbH, Bissendorf, Germany). The botanical pesticide emulsion was shaken before spraying it on the beetles and on the food (except apple pieces, which were added later). Because smaller boxes were used for the beetles, only 2.9 ml of the botanical pesticide formulation was sprayed in each box (equal to three pump sprays). After spray treatment, the boxes were closed with a plastic cover (lacking pores). This experiment was repeated four times. Every second day, a small piece of an apple was provided as fresh food. The number of living and dead individuals was counted after gently shaking the boxes.

Effect of the botanical pesticide on the feeding activity

The effect of the botanical pesticide on the feeding activity of adult desert locusts (*S. gregaria*) was investigated in a separate experiment where the same amount of grass (36 stalks) was added to three boxes of dimensions 20 × 12 × 14 cm. Each box contained 10 adult locust individuals and a small piece of paper towel (one layer of kitchen paper tissue of dimensions 12 × 12 cm). One box served as the no-treatment control. Locusts in the second box were sprayed together with the grass (4.9 ml of the botanical pesticide formulation). Desert locusts in the third box were sprayed with same amount of the botanical pesticide, but fresh grass stalks were added right after treatment. 24 h later, images were taken from each box (see Fig. S2).

Ambient conditions during experiments

All experiments were performed on a work bench with medium airflow and a light/dark cycle of 12:12 h. During the experiments, the temperature was 24.5 ± 2 °C (mean ± SD), and the relative humidity was $42 \pm 11\%$ (mean ± SD).

Effect of the botanical pesticide on plant growth

A botanical pesticide that is based on hardening linseed oil may adversely affect plant growth because of its viscosity. In order to study the potentially harmful effect on plant growth, the botanical pesticide emulsion was sprayed on wheat seedlings that were exposed to sunlight on a balcony in Graz. In this experiment, 4.9 ml of the botanical pesticide emulsion (equal to five pump sprays) was sprayed on each grass pot and grass growth was monitored over 22 days. The wheat seedlings were purchased as “cat grass” from a pet store (Zoo Muser, Graz, Austria). The temperature during this experiment was between 17–30 °C, and the relative humidity was 49.2–62.7%. Each control and treatment group consisted of three pots of wheat seedlings that were submersed in water to provide the same amount of water.

Mortality rate calculations

The mortality rates were calculated in Microsoft Excel 2016 using Eq. (1):

$$\text{Percentage observed mortality} = \left(\frac{\text{total number of dead individuals}}{\text{total number of treated individuals}} \right) * 100 \quad (1)$$

According to the World Health Organization's (WHO) test procedures for insecticide resistance monitoring (Global Malaria Programme 2016), Eq. (2) was used for the calculation of mortality rates that takes the value of the control mortality into account. If the control mortality is higher than 5% and lower than 25%, then the mortality rate of treatment groups was corrected according to the Schneider-Orelli formula (Püntener 1981) shown in Eq. (2).

Percentage corrected mortality

$$= \left(\frac{\% \text{ observed mortality} - \% \text{ control mortality}}{100 - \% \text{ control mortality}} \right) * 100 \quad (2)$$

The corrected mortality was calculated for ladybirds and mealworm beetles, since the mortality of locusts in the control group was less than 5%. To cross-check the corrected mortality rates, we calculated them a second time using the Schneider-Orelli method provided on this website: <http://www.ehabsoft.com/ldpline/onlinecontrol.htm>. The average mortality is given as the mean mortality rate of different replicates ($\pm$ standard deviation).

Statistical analysis

Mortality rates of the locusts and beetles were tested for statistically significant differences by performing the Z-test with the Yates correction, which takes the sample number into account. Significant differences between the control and the treatment groups are indicated by an asterisk in Figs. 2, 3 and 4. All statistical tests were performed in Sigma Plot version 13 (Systat Software Inc.). The linear regression performed for the calculation of the LC 50% of the botanical pesticide was calculated in Excel (version 2016, Microsoft Inc.). To determine whether a possible

synergistic toxic effect was caused by a mixture of four oils, we calculated the expected mortality rate according to what is called the response addition or Bliss Independence (Bliss 1939) and compared this rate with the observed mortality rate by performing a Chi square test (equation described in Tounou et al. 2008). The expected mortality rate caused by the addition of the toxicity of four oils was calculated by modifying equation two as stated in Ceder-green (2014).

Results

Screening for effective essential oils

In a petri dish, the linseed oil/bicarbonate emulsion (56:44% (v/v), Fig. 1a) hardened within 24 h and became sticky and viscous (compare Fig. 1a with 1b). This emulsion caused a moderately toxic effect when sprayed on adult desert locusts (Fig. 2), whereas spraying either linseed oil or bicarbonate solution failed to harm these insects. Linseed oil/bicarbonate-treated desert locusts that survived 24 h had difficulties moving quickly and were unable to jump; they appeared as though frozen. The amount of oil sprayed in this screening experiment was too low to create a sticky surface inside the box upon which insects adhered. To enhance the toxic effect caused by the hardening of linseed oil (see Fig. 2), different essential oils were added to this oil emulsion and sprayed on desert locusts in the screening experiment. Figure 2 shows the mean mortality rates of the desert locust 1 day after a single spray treatment with each of nine different oil emulsions. The mortality rates of desert locusts were equal or exceeded 70% when each of the garlic, orange, caraway and

Fig. 2 The mortality rate of desert locusts in the screening experiment. The mortality rate of the desert locusts is shown after 24 h ($N=10$). *Indicates the significant difference between controls and treatment ($p < 0.05$, Z-test)

Fig. 3 Botanical pesticide experiment. Essential oils were added either individually or in combination to the linseed oil emulsion and the mean mortality rate of desert and migratory locusts was calculated after a single spray treatment. The mean mortality rate of desert locusts (blue bars, $N=10$, five replicates) and migratory locusts

(blank bars, $N=10$, five replicates) is shown after 24 h. *Indicates a significant difference between control 1 and treatment ($p < 0.05$, Z-test). †Indicates a significant difference between control 2 and treatment ($p < 0.05$, Z-test)

Fig. 4 Beetle experiment. The mortality rates of two beetle species after single spray treatment with the botanical pesticide formulation. The mean corrected mortality rate of mealworm beetles (green bars, $N=16$, four replicates) and the mean corrected mortality rate of ladybird adults (blank bars, $N=30$, four replicates) 8 days after treatment. *Indicates a significant difference between control and treatment ($p < 0.05$, Z-test)

birch oils were added at a concentration of 5.3% to the linseed oil emulsion. The highest mortality rate was found after adding garlic oil. Using the same concentration of clove, peppermint and basil oil emulsions also led to significantly higher mortality rates of desert locusts as compared to the controls ($p < 0.05$, Z-test). In contrast, the use of 5.3% ginger or eucalyptus oils did not significantly increase the toxicity of the linseed oil emulsion.

Combination of essential oils

Each of the garlic, orange peel, caraway and birch oils mixed with the linseed oil emulsion caused high mortality in the screening experiment. At lower concentrations,

caraway (2.6%), orange peel (1.3%) and wintergreen (1.3%) oils resulted in a rather low mortality of desert locusts after spray treatment. However, the combination of these oils in the botanical pesticide treatment increased the mortality rate of desert locusts significantly compared to all other treatments and the control situation. This botanical pesticide was more effective against migratory locusts (blank bars in Fig. 3) compared to desert locusts (blue bars in Fig. 3), where $20 \pm 7.1\%$ survived the first day after spray treatment. However, surviving individuals were rather weak and died within in the next 16 h (for an example see Figure S2c, d). The expected additive mortality rate caused by four oils was 56% in *Sch. gregaria* and 216% in *L. migratoria*. The result of the Chi-squared test was 11.7 when we compared the

expected with the observed mortality of *Sch. gregaria*. The critical value of this test defined for three degrees of freedom and an error probability of 0.05 is 7.8. Since the result of the Chi-squared test is higher than this critical value, a synergistic toxic effect caused by the combination of these oils was evident in *Sch. gregaria*. The essential oils evaporated and left no trace of scent behind when we counted the number of dead individuals 24 h after spray treatment. In contrast to the combined effect, spraying only the linseed oil emulsion without essential oils (control 2) had only a minor toxic effect on desert locusts ($14 \pm 16.7\%$ mortality), but still killed $46 \pm 19.5\%$ of migratory locust individuals, which were also more sensitive to individual essential oil treatments.

LC₅₀ test and antifeedant effect

The lethal concentration (LC₅₀) of the botanical pesticide formulation observed 40 h after spraying desert locusts was calculated from the regression line shown in Fig S1. At a concentration of 60%, this botanical pesticide still killed 50% of desert locust individuals, which demonstrates that the toxicity of this botanical pesticide is high enough to control desert locusts effectively. To test for the additional oral toxicity of this botanical pesticide, we sprayed either desert locusts together with grass stalks or added grass stalks after the spray treatment. As can be seen in Figure S2b, the untreated locusts ate all the grass within 1 day. In fact, the desert locusts refused to feed on grass whether it was sprayed or not (Fig. S2c and d). This demonstrates a strong antifeedant effect of this botanical pesticide.

Botanical pesticide experiment performed with two beetle species

To collect preliminary data about the effect of this botanical pesticide on a beneficial and an alternative non-target species, we sprayed this formulation on ladybirds and on adult mealworm beetles (Fig. 4). Spraying adult mealworm beetles (i.e., the alternative non-target species) did not increase their mortality rates significantly. Even 8 days after treatment, the mean corrected mortality of mealworm beetles was only $12.4 \pm 7\%$ (green bars in Fig. 4, $p > 0.05$, Z-test, $N = 16$, four replicates). However, spraying ladybird adults (i.e., the beneficial non-target species) caused a significant mean mortality rate of $67.7 \pm 8.9\%$ as compared to the control group after 8 days (blank bars in Fig. 4, $p < 0.05$, Z-test, $N = 30$, four replicates). Ladybird larvae were even more sensitive because $56.7 \pm 4.7\%$ of all individuals died within 1 day after the botanical pesticide treatment ($p < 0.05$, Z-test, $N = 15$, two replicates). Records on the effect of the botanical pesticide on the ladybird larvae could not be taken after the first 24 h, due to the fact that the larvae cannibalized each other.

Effect of the botanical pesticide on grass growth

We sprayed the botanical pesticide on fresh wheat seedlings to investigate its impact on plant growth. We investigated the growth of the wheat seedlings that were exposed to natural sunlight on a balcony (Fig. 5a). Figure 5 shows the pots of the wheat grass 22 days after treatment. Almost no difference was observed in the shape and growth of grass stalks between the control (Fig. 5b) and the treated grass (Fig. 5c), with the exception of some yellow tips.

Discussion

Due to the hardening process of the linseed/bicarbonate emulsion (see Fig. 1), locust mobility was strongly restricted after 24 h. This was obvious as individuals rarely jumped and moved slowly after disturbance. Adding sodium bicarbonate to the linseed oil clearly accelerated the hardening process of this oil. Bicarbonate salts are also known to be effective against fungal plant diseases. For example, Kuepper et al. (2001) provided a brief survey of the use of sodium bicarbonate (NaHCO₃) and potassium bicarbonate (KHCO₃) to control powdery mildew and other fungal diseases. Similarly, Horst et al. (1992) used sodium bicarbonate to control powdery mildew and black spot disease in roses in greenhouses and field experiments. However, spraying the linseed oil/bicarbonate emulsion on desert locusts was not very effective, since only 40% of treated individuals died within 1 day (Fig. 2). To enhance the harmful effect of this linseed oil emulsion, several essential oils were added in a screening experiment. The results of this experiment revealed that garlic, caraway, orange and birch oil emulsions were potential candidates for an insecticidal oil mixture that could kill a high proportion of locusts within a short time period. The desert locust mortality caused by these oils applied at concentration of 5.3% was equal or even exceeded 70%. However, garlic oil was excluded from the next experiment because of its strong and unpleasant smell. Furthermore, birch oil was replaced by wintergreen oil, because wintergreen is more readily available and has the same active ingredient (methyl salicylate).

A formulation consisting of linseed oil and low concentrations of orange peel, wintergreen and caraway oils was then sprayed on desert locusts and migratory locusts to test its potential as a novel botanical pesticide. Results shown in Fig. 3 demonstrate that the combination of these essential oils had a strong effect on the mortality rates of the two gregarious locust species. Desert locusts were less sensitive as compared to migratory locusts, but individuals surviving the first 24 h died within the next 16 h. Although the use of the same concentration of each essential oil had only a low or moderate effect on desert and migratory locust mortality, the

Fig. 5 Spray treatment of wheat seedlings with the botanical pesticide emulsion. **a** Images of wheat seedlings before treatment. **b** Image of the untreated wheat grass taken 22 days later. **c** Image of the treated wheat grass 22 days after spray treatment

combination of the three essential oils resulted in a strong toxic effect. The results obtained with desert locusts demonstrate that these essential oils act synergistically, because the high mortality rate of desert locusts treated with the botanical pesticide cannot be explained simply by an additive toxic effect caused by each essential oil (Fig. 3).

The botanical pesticide formulation developed in this study harmed the target insects in at least two ways: First, the mobility and most likely the ventilation of the locusts were affected due to the hardening of the linseed emulsion (see Fig. 1). Second, an antifeedant effect was clearly observed, since all locusts stopped feeding immediately after the spray treatment (see Fig. S2). These results, taken together, indicate that our botanical pesticide formulation constitutes a novel, promising and effective agent that can be used to combat upsurges and locust outbreaks. This botanical pesticide formulation was then tested on two beetle species in an experiment lasting for 8 days (Fig. 4). Both beetle species are related to ecologically important beetle species (Jankielsohn 2018). Within this time span, although mealworm beetles (i.e., alternative non-target species) behaved normally, a significant, toxic effect was observed in ladybird adults (i.e., beneficial non-target species). Even more problematic a strong toxic effect was observed in the ladybird larvae that were studied for just 1 day after treatment. Mealworm beetles are less likely to come in contact with our botanical pesticide in the field, except when locusts are treated in the Mediterranean region, which is the home of

this beetle species. However, given the fact that the ladybird species used in this study is related to other ladybird species in various ecosystems we cannot exclude an unwanted side-effect on non-target insect species with important ecological functions (e.g., feeding on plant lice). However, this botanical pesticide may have only minor negative effects on the vegetation, since the growth of wheat seedlings on a balcony was not significantly affected (Fig. 5). It will be possible to use conventional spraying devices for field applications of this botanical pesticide when the emulsion is stabilized by means of an emulsifier. To avoid a toxic effect on non-target species, such as other insects (e.g., bees, beetles) and birds, it is highly recommended to spray the pesticide after sunset when the locusts rest in crowded colonies on bushes and trees.

To understand the synergistic toxic effect caused by this botanical pesticide, it is necessary to focus on its active ingredients. The main component of orange peel oil is limonene (87.9–92.5%; Njoroge et al. 2009), but caraway oil also contains this cyclic monoterpene (1.5–51.3%; Raal et al. 2012). A recent study demonstrated that the ventilation in the migratory locust is adversely affected by limonene in its pure form or as the major component of lovage essential oil (Halawa and Hustert 2014). Wintergreen oil mainly contains methyl salicylate (97–99.6%; Gurung 2007), which was found to have insecticidal activity against the adzuki bean weevil *Callosobruchus chinensis* (Park et al. 2016). Raal et al. (2012) identified carvone (44.5–95.9%) as the main component of

caraway oil, and Wawrzyniak and Lamparski (2006) found that aqueous extracts of caraway inhibited the feeding activity of the Colorado potato beetle *Leptinotarsa decemlineata* and its larvae. This suggests that caraway may be responsible for the antifeedant effect found in locusts. Additional studies, such as the one performed on the cabbage looper (*Trichoplusia ni* Hübner, Noctuidae, Akhtar et al. 2012), are necessary to reveal the mechanism behind the antifeedant effect of our botanical pesticide. The insecticidal effects of caraway and wintergreen oils on locusts shown in this study is novel and have not been described before.

The main component of the botanical pesticide formulation is the linseed oil, which contains omega-3 fatty acids and has a higher linolenic acid content than other plant oils. For this reason, linseed oil is healthy and may be used as an alternative to fish oil in the human diet (Kolodziejczyk et al. 2012). Since locusts are collected and consumed as a protein source in some countries in Africa, Asia, Central and South America, Australia (Paul et al. 2016), locusts that have been stunned or killed by our botanical pesticide can be collected and used as a protein source in the human diet and the food industry. The other components of this botanical pesticide formulation are often used as preservatives and biological agents in many applications and in alternative medical treatments for human, animals and plants (Elshafie and Camele 2017).

Linseed oil becomes viscous over time when mixed with the bicarbonate solution, but it may also have an additional adverse effect on locust swarms due to the unsaturated fatty acid content. Yao et al. (2009) reported that certain unsaturated fatty acids like oleic and linoleic acid constitute necromones that are released by dead insect bodies. Necromones can be recognized by other individuals of the same or different species, where they evoke distinct behavior. For example, social insects remove the dead bodies from their nests (necrophoric behavior), whereas semi-social species avoid contact with dead or injured individuals (necrophobic behavior). Therefore, Yao et al. (2009) suggested that fatty acid necromones might be considered in many contexts, including pest management. Linseed oil contains approximately 9–11% saturated (5–6% palmitic acid and 4–5% stearic acid) and 75–90% unsaturated fatty acids (50–55% linolenic acid, 15–20% oleic acid; Bayrak et al. 2010), which suggests that this oil may evoke necrophobic behavior in swarm mates. This might disrupt swarm formation after a certain percentage of individuals have come into contact with linseed oil. Current studies aim to reveal whether linseed oil has a possible swarm disrupting effect.

In conclusion, we were able to develop a potent novel oil mixture consisting of linseed oil and plant essential oils that displayed toxicity against locusts. This botanical pesticide contains caraway, wintergreen and orange peel oils at low concentrations. Due to a combined toxic effect, it is effective

against two species of gregarious locusts (Fig. 3) while it did not harm mealworm beetles. Due to the toxic effect on the ladybird adults (Fig. 4) and its highly toxic effect on their larvae, the application of this botanical pesticide should be restricted to dense locust colonies where other non-target insects are rare. It was interesting to note that our botanical pesticide formulation did not adversely affect the growth of wheat seedlings (Fig. 5). These results suggest this formulation is an effective botanical pesticide that can be used as an alternative to chemical pesticides in the future. The components of our botanical pesticide formulation can be obtained easily at low prices, and no laboratory equipment is needed to mix the ingredients before the pesticide is applied. However, further research under field conditions is necessary to determine the effects of this novel insecticidal formulation on locust swarms and the local ecosystem.

Author contributions

ZASA and MH conceived and designed the research. ZASA conducted the experiments. ZASA analyzed the data and drafted the manuscript. MH supervised the experiments and critically read the manuscript. All authors read and approved the manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The experimental procedures were approved by the Institute of Biology (University of Graz). All experiments comply with the current Austrian and European Community laws for the ethical treatment of animals and are in line with the ASAB Guidelines for the Use of Animals in Research.

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