Bioactivity and physico-chemistry of garlic essential oil nanoemulsion in tomato

This is the peer reviewed version of the following article: Ricupero, M., Biondi, A., Cincotta, F., Condurso, C., Palmeri, V., Verzera, A., ... & Campolo, O. (2022). Bioactivity and physico-chemistry of garlic essential oil nanoemulsion in tomato. *Entomol. Gen*, *42*, 921-930. DOI:10.1127/entomologia/2022/1553

Bioactivity and physico-chemistry of garlic essential oil nanoemulsion in tomato

Short title: Bioactivity of garlic essential oil nanoemulsion

Michele Ricupero[1], Antonio Biondi[1], Fabrizio Cincotta[2], Concetta Condurso[2], Vincenzo Palmeri[3], Antonella Verzera[2], Lucia Zappalà[1*], Orlando Campolo[3]

[1]University of Catania, Department of Agriculture, Food and Environment, via Santa Sofia 100, 95123, Catania, Italy

[2]University of Messina, Department of Veterinary Science, Polo Universitario SS Annunziata 98168, Messina, Italy

[3] University of Reggio Calabria, Dipartimento di AGRARIA, Loc. Feo di Vito, 89122, Reggio Calabria, Italy

*corresponding author

e-mail lzappala@unict.it

Phone +390957147258

Abstract

Tomato has an economic relevance worldwide but its production is threatened by several biotic factors, including the invasive South American tomato pinworm *Tuta absoluta*. The control of this pest mainly relies on the repeated applications of synthetic insecticides that can have considerable non-target effects; therefore, new sustainable approaches are required. The biocidal activity of garlic has been recognized and no risks for consumers and the environment are expected in its use. However, the practical implementation of garlic extracts is hampered by several draw backs that could be overcome by nanotechnologies. We developed and characterized a new garlic essential oil-based nanoemulsion (GEO-NE) and laboratory trials were carried out to investigate its insecticidal activity against *T. absoluta* involving different instars and exposure routes. GEO-NE side effects on the mirid predator *Nesidiocoris tenuis* and tomato plants were also assessed in the laboratory. The nanoformulation had dimensions belonging to the nanometric scale and good stability over time. GEO-NE showed significant toxicity toward *T. absoluta* eggs and larvae and repellence for ovipositing females. No lethal effect on *N. tenuis* adults was recorded but its progeny was significantly reduced on GEO-NE treated plants. By contrast, GEO-NE had no phytotoxic effects on sprayed tomato plants. Our findings suggested that GEO-NE can successfully control *T. absoluta* and its application deserves to be considered as a potential tool for tomato Integrated Pest Management.

Keywords: biopesticide, phytotoxicity, oviposition deterrence, botanicals, nanoinsecticide, selectivity

Introduction

Intensive agriculture is heavily reliant on pesticides for food protection but these chemical substances pose adverse impacts on human health, water quality and biodiversity on a global scale (Tang et al. 2021). For these reasons, public opinion and policymakers strongly encourage sustainable practices for pest control aiming at food safety and food security (Carvalho et al. 2006). Among botanical insecticides, plant essential oils (EOs) are considered environmentally friendly control tools mainly due to their rapid biodegradability, low risks of resistance phenomena and negligible toxicity towards non-target organisms (Regnault-Roger et al. 2012). Because of this promising evidence, botanicals have been regarded as a panacea for pest concerns over the last two decades (Campolo et al. 2014; Pavela & Benelli 2016; Galland et al. 2020; Pavela et al. 2020). Despite the massive body of literature produced, poor studies corroborate EO practical implementation which is limited by their constitutive drawbacks, e.g., stability and degradation patterns, changing toxicity towards the target and non-target organisms (Isman 2020). Nevertheless, nanotechnology could help overcome the intrinsic constraints often associated with the use of EOs (Athanassiou et al. 2018; Campolo et al. 2020a; Pavela et al. 2021; Sciortino et al. 2021).

Garlic, *Allium sativum* Linnaeus (Amaryllidaceae), is a commercial crop widely cultivated around the globe and China is its largest exporter worldwide (Rabinowitch & Currah, 2002). The long-standing use of garlic as food spice and medicine throughout human history has been associated with anticancer, cardiovascular and biocidal activities (Thomson & Ali 2003). The latter has been demonstrated in the laboratory against different pests including insects, mites and nematodes (Park et al. 2006; Vergel et al. 2011; Palermo et al. 2021). Although the non-target impact of garlic on beneficial arthropods is mostly unknown (Asadi et al. 2019), a recent pesticide peer-review published by The European Food Safety Authority (EFSA) recognizes no risk to consumers in the use of garlic as a plant protection product (Anastassiadou et al. 2020).

Tomato crop has a very high social and economic relevance worldwide and the South American tomato pinworm, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), negatively affected the entire cropping system in Palaearctic, Afrotropical and Indomalayan realms during the last decade (Biondi et al. 2018). Synthetic insecticides are the most used control tool against this pest, but a plethora of adverse consequences have been continuously reported in their use (Desneux et al. 2007; Guedes et al. 2019; Soares et al. 2019a). Sustainable control tactics against *T. absoluta* have been developed across different world regions with promising results, but control failures by chemical pesticides and the high cost of biological and biotechnical solutions remain the biggest challenges for tomato growers worldwide (Desneux et al. 2022).

In previous researches, the use of EO-based insecticides against *T. absoluta* was assessed with promising results in both laboratory and field-applications (Campolo et al. 2017; Mansour & Biondi 2021; Desneux et al. 2022). Similarly, the non-target impact of EOs on the pest predator *Nesidiocoris tenuis* (Reuter) (Hemiptera: Miridae) was recently studied (Soares et al. 2019b; Campolo et al. 2020b). However, the toxicity of garlic EO on this biological system has not been investigated. Here, we tested garlic EO as insecticide against *T. absoluta* involving different instars and exposure routes. The egg was our first target stage since it is considered the least susceptible instar to both chemical and naturally-derived substances (Goudarzv Chegini & Abbasipour 2017; Campolo et al., 2017; Tomè et al., 2012). The LC_{50} estimated for *T. absoluta* eggs and the maximum tested concentration were evaluated as larvicidal and oviposition deterrent. Further experiments were also addressed to evaluate the side effects of garlic-EO-based nanoformulation towards the aforementioned biological model. Our results can contribute for implementing sustainable control strategies of *T. absoluta* in the tomato cropping system.

Materials and methods

GC-MS analysis and Nanoemulsion preparation

Pharmaceutical grade *Allium sativum* (thereafter Garlic) EO was purchased by Esperis s.p.a. (Milan, Italy). The sample was diluted 1:100 with n-hexane and analysed with a Shimadzu GC 2010 Plus gas chromatograph coupled with a TQMS 8040 triple quadrupole mass spectrometer equipped with a DB-5ms, 30 m, 0.25 mm i.d., 0.25 µm film thickness non polar column (Supelco Sigma-Aldrich, Bellafonte PA, USA). The following conditions were used: injector temperature, 250 °C; injection mode, split; split ratio, 1:100; oven temperature, 40 °C held for 2 min, then increased to 110 °C at a rate of 4 °C/min and to 240 °C at a rate of 3 °C/min and held for 3 min; carrier gas, helium at a constant flow of 1 ml/min; transfer line temperature, 240 °C; ionization technique; electron impact (EI) at 70 eV; acquisition range, 40 to 400 m/z; scan rate, 3 scan/sec.

The identification of volatile compounds was conducted according to Cincotta et al. (2021). Quantitative results were expressed as average peak areas of 3 replicates.

The Garlic EO-nanoemulsion (GEO-NE) was prepared using the self-emulsifying process followed by sonication according to the methodology described by Campolo et al. (2020a). The average droplet size and size distribution (Poly dispersion index), were measured by using a dynamic light scattering particle size analyser (Z-sizer Nano, Malvern Instruments) at 25 °C. In addition, the particle surface charge was quantified as zeta potential (ζ) using a Z-Sizer Nano, (Malvern Instruments) at 25°C. Changes in droplet size and ζ were measured over time up to 16 weeks after the nanoemulsion preparation.

Biological materials

Tomato plants (*Lycopersicon esculentum* Mill., Solanaceae) used for both insect rearing and experiments were grown in greenhouse conditions in 1L pots, inside screened cages without pesticide application. *Tuta absoluta* laboratory rearing was established and maintained as described by Campolo et al. (2017). *Tuta absoluta* eggs and larvae of the same age were obtained by releasing about two hundred newly-emerged adults inside each cage containing four tomato plants when they reached the phenological stage of 3rd leaf on the main shoot unfolded. The moths were left 24 h to lay eggs and then removed. Eggs (72 ± 12 h old) and newly-molted 2nd instar larvae were used for the bioassays. The *N. tenuis* colony was established and kept in the laboratory as described by Passos et al. (2022). Newly emerged (1-4-day-old) adults of *N. tenuis* were collected from the rearing cages by a mechanical aspirator, coupled in plastic tubes and kept refrigerated (∼7°C) until their use.

Bioassays

The following bioassays were carried out at the Department of Agriculture, Food and Environment of the University of Catania (Italy) in climatic chamber under controlled environmental conditions (25 ± 2 °C, 60 ± 10% RH, 14:10 L:D). The tested GEO-NE solutions were prepared by mixing the necessary amount of concentrated nanoemulsion (15% of EO) with distilled water in order to obtain the required concentration for the different bioassays. Because the developed nanoemulsion was able to disperse easily in water, a slight stirring (10 sec at 2,000 RPM) by means of a magnetic stirrer was needed for preparing the solutions.

A spinosad-based commercial insecticide (Laser™ Dow Agrosciences, applied at double highest label rate recommended in Italy for tomato crops, 150 mL/hL) was used as treated control in the bioassays involving *T. absoluta* because its use is widely recognized in Mediterranean basin organic tomato cultivation (Biondi et al. 2018). For the assessment of non-target impact towards *N. tenuis*, an indoxacarb-based insecticide (Steward®, DuPont™, applied at the highest label rate recommended in Italy for tomato crops, 12.5 g/hL) was used since this active ingredient has been recognized as harmful towards the predator in laboratory condition s (Arnò & Gabarra 2011). Distilled water and TWEEN® 80 + distilled water were used as untreated controls.

Toxicity toward *T. absoluta* juveniles

Two bioassays were carried out for evaluating the efficacy of the developed formulation to control *T. absoluta* egg and larval stages, respectively. In the first bioassay, tomato plants bearing *T. absoluta* eggs, obtained as described above, were sprayed 72 hours later with seven different concentrations (from 0.015 to 3% W/W) of GEO-NE formulation until runoff by using a 2 L power-pack aerosol hand sprayer (Dea®, Volpi, Italy) and left to dry for one hour. For each replicate, ten sprayed *T. absoluta* eggs were carefully transferred on untreated tomato leaves through a fine paintbrush inside a ventilated arena (Biondi et al. 2012). The egg mortality was daily checked up to 48 hours after egg hatching.

In the second bioassay, tomato plants were sprayed with both the resulting$LC_{50\ eggs}$ and the maximum concentration tested against the egg stage (i.e., 3% of EO). These two concentrations were chosen for assessing the potential larvicidal activity that can be simultaneously determined by the ovicidal treatment. For each replicate, ten coetaneous 2nd instar *T. absoluta* larvae were transferred to sprayed tomato leave inside a ventilated arena according to the methodology described by Campolo et al. (2017). Larval mortality was assessed 24 and 72h after the spray. Non-reacting larvae when stimulated with a fine paintbrush were considered dead. Chronic toxicity was assessed by calculating the proportion of juveniles, alive 72 h after the spray, that reached the adult stage. Consequently, 14 and 12 days after exposing larvae to the chemicals, the isolators were checked daily to record adult emergence. Cumulative mortality (acute and chronic) was used to evaluate the efficacy of the developed formulation. Both bioassays were replicated five times for each tested concentration and the controls.

Oviposition deterrence

Choice and no-choice tests were carried out to evaluate the oviposition deterrence on *T. absoluta* adult females caused by the ovicidal treatments. To obtain coetaneous and mated females, *T. absoluta* pupae were sexed and, once adults had emerged, 5 females and 5 males were coupled and allowed to mate for 4 days. No oviposition substrate was provided during this period.

In both experiments, tomato plants were sprayed with the resulting LC_{50} for eggs and left to dry. Only distilled water + Tween 80 was used as a control since no statistical difference ($p>0.05$) was recorded between this treatment and water alone (data not shown) in preliminary trials.

In the no-choice test, two sprayed tomato shoots with the base immersed in water, were placed inside a polyester net cage (50 × 60 × 80 cm), whereas in the choice-test, both treated and control shoots were placed inside the cages. Ten *T. absoluta* adults (5 females and 5 males) were released in the cages and maintained in the same climatic conditions described above. After 72 h, the number of eggs laid both in the treated and control shoots were counted by using a stereomicroscope. Each experiment was replicated ten times.

Side effects on *Nesidiocoris tenuis* and tomato plants

To evaluate the side effects of *T. absoluta* ovicidal treatments towards *N. tenuis,* two different experiments were carried out, which aimed at evaluating the residual toxicity of GEO-NE on the survival and the progeny production of the predator. Shoots were collected 1h and 72 h later from tomato plants sprayed with $LC_{50\ eggs}$, the highest application rate (3% of EO) used in the ovicidal bioassay, treated and untreated controls as described above. Also in this case, only distilled water + Tween 80 was used as a control since water alone had no effect on *N. tenuis* mortality and its progeny production ($p>0.05$). Five couples of *N. tenuis* were released inside the above-described isolator provided with a sprayed shoot (1h or 72-h-old residues) and devitalized *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs as a food source. Mortality was assessed daily for three days by recording the number of alive and dead adult males and females. After three days, adults were removed and ten days later the number of nymphs was recorded daily for additional ten days. Each treatment was replicated ten times.

The toxic effect of the developed formulation on tomato plants was evaluated for two weeks by spraying five additional tomato plants with the seven application rates described above, following the methodology described in Campolo et al. (2017). Control treatments (i.e. indoxacarb, water + Tween 80 and water alone) were also included.

Data analysis

Mortality data were corrected for control mortality using the Abbott's formula (Abbott, 1925). Dependent variables were tested for homogeneity and normality of variance (Levene and Shapiro-

Wilk test respectively) and transformed (arcsin √x) whenever needed. Probit analysis was performed in order to estimate the median lethal concentrations (LC_{50} and LC_{90}) with associated 95% confidence intervals. Values were considered significantly different when their 95% fiducial limits did not overlap. Mortality and oviposition data in choice test, and progeny production, were subjected to univariate analysis of variance following the GLM procedure. Choice test data was subjected to the χ^2 goodness of fit analysis to test the null hypothesis that oviposition was not influenced by the treatment (response equal to 50:50). Multiple comparisons were carried out using Duncan’s multiple range post-hoc test. To evaluate the effect on plants of the developed formulation, the Phytotoxicity index (P_i) was calculated according to the formula proposed by Campolo et al. (2017).

$$P_i = \sum_{j=0}^{n} \left(\frac{DLj}{TL} X \frac{DC}{n-1} \right)$$

where DL is the number of damaged leaves for each damage severity class j, TL is the total number of leaves sprayed, DC is the damage severity class, and n is the number of damage severity classes. The Pi ranges from 0 (no damage) to 1 (dead leaves).

Results

GC-MS analysis and Nanoemulsion preparation

More than 70 volatile compounds were detected in the Garlic EO with more than 90% referred to sulphur compounds. Diallyl sulphides, from mono- to hexasulfide, quantitatively prevailed (Table S1). In particular, in our samples diallyl disulfide (29.66%) and diallyl trisulfide (21.50%) prevailed, diallyl tetrasulfide (13.19%) and diallyl sulfide (10.69%) followed. Other thiosulfinates, including allyl methyl-, allyl 1-propenyl and methyl 1-propenyl di-, tri-, and tetrasulfides have been identified in small amount in the samples analysed.

The GEO-NE particles had dimensions belonging to the nanometric scale (176.23±0.9 nm) and a surface charge (ζ potential) of -23.16±0.29 mV. The size distribution of the formulation (0.18) highlighted a close distribution of particle size in the analysed samples. Over time (Fig. 1), both the size and the ζ potential increased still reaching values below 183 nm and -18 mV, respectively. During the first three weeks, the particle size remained almost constant (176.23±0.88 nm) and only eight weeks after preparation a small increase in size was measured.

Toxicity towards *Tuta absoluta* juveniles

The mortality of eggs sprayed with the developed formulation had a concentration-dependent response with a value of LC_{50}= 0.124% of EO (CI = 0.098-1.151) and a LC_{90}= 0.772% of EO (CI= 0.601-1.052) as estimated by the probit. analysis (Slope± SE = 1.61± 0.11; $\chi^2$84.612; p =0.084). Conversely, in the water control only 4±1.63%of eggs did not hatch. Statistical differences were highlighted among the egg corrected mortality registered in the GEO-NE treatments compared to the control treated with spinosad (F = 81.933; df = 7; p < 0.001). Our nanoemulsion at 1.5 and 3% of EO concentrations killed 96.88 and 97.92% of the sprayed eggs, respectively. These results were similar to the spinosad-based control (Fig. 2). The formulation showed also promising larvicidal activity. Both the GEO-NE tested concentrations (i.e., 3% and $LC_{50\ eggs}$) caused 100% and

77.78±13.61% mortality of *T. absoluta* larvae, respectively. The GEO-NE efficacy was thus comparable to the spinosad-based control (F=2.667; df = 2; p=0.11).

Oviposition deterrence

In the choice test (Fig. 3), *T. absoluta* females oviposited significantly more eggs on control plants in comparison to plants sprayed with GEO-based formulation (χ^2= 8.601; p<0.01). The mean number of eggs laid per female on control tomato shoots (5.75±1.39) doubled the amount of eggs laid on the GEO-NE sprayed shoots (2.08±0.6). In the no-choice test, untreated shoots resulted more attractive than GEO-NE sprayed shoots (F=90.556; df=1; p=0.01). Namely, the oviposition by *T. absoluta* females was significantly reduced on plants sprayed with the formulation in comparison to control plants (Fig. 3).

Side effects of GEO-NE

The overall mortality of *N. tenuis* caused by GEO-NE was affected by the age of the residues on tomato shoots (F=6.038; df=1; p=0.01). As expected, 1-h-old indoxacarb sprayed tomato plants negatively affected the survival of *N. tenuis* more than GEO-NE at both tested concentrations (F=44.431; df=2; p<0.001) (Table 1). Similarly, on the 3-day-old treated shoots, the mirid mortality was significantly higher for indoxacarb compared to both GEO-NE concentrations (F=148.816; df=2; p<0.001). In 1-h-old residue sprayed tomato shoots, indoxacarb and GEO-NE at 3% killed more than 80% and 60% of the exposed individuals, respectively. Conversely, only ~ 3% of dead *N. tenuis* individuals were recorded after the exposure to1h GEO-NE residues at LC_{50} estimated for *T. absoluta* eggs (F=27.356; df=2; p<0.001).

The progeny produced by *N. tenuis* females was significantly affected by GEO-NE (F=72.150; df=3; p<0.001). The offspring recorded for *N. tenuis* females exposed to 1-h-old 3% GEO-NE sprayed shoots was decreased (0.10±0.07) in comparison to the progeny recorded in water sprayed shoots (13.06±1.23) (Fig. 4). In the shoots treated with the $LC_{50\ eggs}$, the number of progeny was 7.34±0.89. Within the same treatment, difference in the mean of progeny produced was observed only in the GEO-NE 3% treated shoots, in which the age of the residues affected the predator reproduction capacity.

Overall, no sign of toxicity was observed on tomato plants sprayed with the tested GEO-NE concentrations during two-weeks (data not shown). Consequently, the P_i was always equal to zero and classified as no-damage.

Discussion

The GEO nanoformulation we developed showed interesting potential for the control of *T. absoluta* and its effectiveness can be attributed both to the essential oil used and the formulation itself. The Garlic EO we used mainly consisted of sulphur compounds which are responsible for the characteristic smell and taste of garlic (Amagase 2006; Satyal et al. 2017; Condurso et al. 2019). Diallyl disulfide and trisulfide, the most abundant compounds found in our samples, represent the main component of commercial garlic oils, in which diallyl trisulfide prevails in fresh garlic oil (Miething 1988; Jirovetz et al. 1992). These two compounds are known to be effective against stored product pests, mosquitoes, diptera sciaridae, termites, psillidae and psocoptera (Huang et al. 2000; Park et al. 2006; Zhao et al. 2013; Liu et al. 2014).

Despite their promising insecticidal properties, EOs used as such present a series of problems mainly related to their chemical characteristics (e.g. poor water solubility, environmental degradation, phytotoxicity, volatility and flammability), therefore the development of nanoformulations is necessary for their use as insecticides under real operating conditions. However, one of the main problems related to the widespread use of these control tools is related to the limited availability of registered nanoformulations depending on the variety of regulatory approval processes about natural derivatives adopted by different Countries. The nanoemulsion we developed helped solve many of EO constraints by enhancing its dispersion in water, reducing its phytotoxicity and, increasing its stability overtime. The low persistence of essential oils and other eco-friendly products, such as *Bacillus thuringiensis*, can represent a limitation, but at the same time it guarantees the consumer about the absence of insecticide residues in foods.

The nanoscale droplet diameters we obtained (i.e., less than 180 nm) likely contributed to the high effectiveness of the insecticide formulation against *T. absoluta*. Decreasing LC_{50s} of permethrin and neem oil have been observed in nanoemulsions at decreasing droplet size (Anjali et al. 2012). Similar results have been reported by Mossa et al. (2018) in comparing the efficacy of both Garlic EO normal-emulsion and nanoemulsion against two eriophyid mites.

The amount of EO loaded, the surfactant-to-oil ratio (SOR) and the preparation method are critical factors in obtaining a stable and effective nanoemulsion (Donsì & Ferrari 2016). In our insecticidal formulation we were able to load 15% of EO with a SOR of 0.33 while ensuring good stability and a small particle size. In comparison to our nanoemulsion, most of the developed EO-based nanoemulsions contain less than 10% of EO while, formulations loading higher EO percentages (10-16.7) often require higher percentages of surfactant (SOR 1-2) or very high energy processes, such as high-pressure homogenization (Donsì & Ferrari 2016).

The obtained results highlighted a good insecticidal activity of the developed formulation in controlling *T. absoluta* preimaginal instars since both the tested concentrations (NE at 3% of GEO and the $LC_{50\ eggs}$) caused high mortality rates in treated larvae. The evaluation of EOs as control tool of *T. absoluta* is still at an early stage probably because larvae feed on mesophyll tissues and EOs are not able to penetrate up to these tissues if not applied as nanoinsecticides (Campolo et al. 2017). Some researches tried to evaluate the fumigation efficacy of EOs against larvae despite, in our opinion, this approach, when applied towards crop pests, could be useful only to understand the potential of the tested EOs since this method is unlikely to be applied in the open field. Conversely, in stored product industry the use of EOs as fumigants could be a viable alternative to synthetic fumigants (Campolo et al. 2014). Garlic EO had strong fumigant activities against *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) adults and on F1 progeny (Yang et al. 2010a) and against *T. castaneum* and *Sitophilus zeamais* (L.) (Coleoptera: Curculionidae) when combined with diatomaceous earth (Yang et al. 2010b). *Elettaria cardamomum* Maton (Zingiberaceae) EO extracted from seed revealed a good fumigation activity against *T. absoluta* 2nd instar larvae inside and outside the leaves (Goudarzv Chegini & Abbasipour 2017) as while *Artemisia absinthium* L (Asteraceae), *Eupatorium buniifolium* Hooker et Arnott (Asteraceae) EOs applied as fumigants (e.g. vapors) and by contact toxicity route (Umpiérrez et al. 2017).

Tuta. Absoluta eggs are considered less susceptible both to EOs and chemical insecticides than larvae (Tomé et al. 2012; Goudarzv Chegini & Abbasipour 2017). Our results confirm this aspect since the lethal concentration that killed the 50% of the eggs was able to kill almost 80% of the treated larvae which feed mainly protected inside the mines. Our results suggest that Garlic EO seem to be more effective against the moth eggs than other EOs. Campolo et al. (2017) evaluating the efficacy of Lemon, Mandarin and Sweet Orange Citrus peel EOs emulsion against *T. absoluta* eggs highlighted that the all the tested concentrations (from 2.5 to 40 mg of EO x mL^{-1}), much higher than that used in this study, were not able to kill the 50% of the treated eggs. LC_{50} value of *E. cardamomum* EO applied as fumigants against *T. absoluta* eggs was significantly higher than that of the 2nd instar larvae inside mines (351.19 vs 7.88 μl L^{-1} air respectively) (Goudarzv Chegini & Abbasipour 2017).

GEO-NE revealed also a good oviposition repellence both in the choice and no choice tests by reducing the eggs laid by female more than 50% on the treated shoots. Oviposition repellence due to EOs in *T. absoluta* was also highlighted by Yarou et al. (2018), by treating tomato plants with *Ocimum gratissimum* L. (Lamiaceae) and *Ocimum basilicum*L (Lamiaceae) EOs (0.5 and 1 mg) formulated with paraffin oil. In addition, the same Authors highlighted a reduction in eggs laid on tomato plants when associated with basil plants which might have masking tomato VOCs and preventing *T. absoluta* females from recognizing tomato plants. In *Tetranychus urtica*e Koch (Acari: Tetranychidae) sublethal concentrations of *Piper marginatum* Jacq (Piperaceae) EO and its major compounds affected the fecundity of females (Ribeiro et al. 2016).

Despite the EOs were largely tested as pesticides, only few studies targeted the adverse impact on plants and non-target organisms (Pavela & Benelli, 2016). In our study, both the tested application rates of the developed formulation ($LC_{50\ eggs}$ and GEO-NE 3%) had an impact on *N. tenuis* adult survival lower than the indoxacarb-based treated control. When the mirid was released 72h after the treatments, the residues had low effects on the predator mortality. Conversely, the developed formulations had an important influence on the mirid progeny production and only at the lowest tested concentration ($LC_{50\ eggs}$) the progeny produced by females was significantly higher in comparison to the treated control. Moreover, the age of residue had negligible effects on the offspring production. Our results suggest that GEO-NE acts, as recorded for *T. absoluta*, as oviposition deterrent since the treatments in which most of females survived (i.e. shoots treated with GEO-NE 3% 72h before the mirid release) the progeny produced by females was similar to that registered in the positive control in which most females died.

Biopesticides are generally considered ecologically-sound since they are thought selective, less threatening to the environment and human health. Despite these beliefs, several studies prove that this issue cannot be generalized. For instance, although the survival analysis of *N. tenuis* predators exposed to citrus oil-based insecticide residues at different concentrations indicated no significant differences from the untreated control (Soares et al. 2019), borax + citrus oil based formulation had the same adverse impacts (mortality and progeny production) of indoxacarb on the predator *Orius laevigatus* (Fieber) (Hemiptera: Anthocoridae) (Biondi et al. 2012).

Umpierrez et al. (2017) reported that *A. absinthium* and *E. buniifolium* EOs were toxic to honeybees when applied at the concentrations effective against *T. absoluta* larvae. Essential oils had negative effects also on the predatory mite *Amblyseius swirskii* Athias-Henriot (Acari, Phytoseiidae) by affecting both the female survival as well as egg laying (Amer & Momen 2002). Conversely, *Piper marginatum* EO applied as fumigant against the two-spotted spider mite *T. urticae* and the generalist mite predator *N. californicus* was the less toxic to the natural enemy than the pest (Ribeiro et al. 2016). Oregano EO and its different compounds affected the survival of green lacewing *Chrysoperla externa* (Hagen) (Neuroptera: Chrysopidae), having often sublethal effects on its fecundity and fertility (Castilhos et al. 2018).

Kimbaris et al. (2010) showed that the coccinellid predators *Adalia bipunctata* L (Coleoptera: Coccinellidae) and *Coccinella septempunctata* L (Coleoptera: Coccinellidae) were 2 to five times more susceptible to *Mentha* spp. EOs applied as fumigant compared to their prey *Acyrthosiphon pisum* Harris (Hemiptera: Aphididae) and *Myzus persicae* Sulzer (Hemiptera: Aphididae); whereas, orange EO had LC values higher in the coccinellids than in the aphids. Also, *Origanum vulgare* and *Thymus vulgaris* L (Lamiaceae) EOs applied as fumigants were selective toward *Trissolcus basalis* (Woll.) (Hymenoptera: Scelionidae) (González et al. 2013). *Piper aduncum* (Piperaceae) EO when applied via contact and immersion routes against *Euschistus heros* (F.) (Hemiptera: Pentatomidae) caused deleterious effects to different stages of the stink bug without effects toward its natural enemies, *Telenomus podisi* (Ashmead) (Hymenoptera: Platygastridae) and *Trissolcus urichi*(Crawford) (Hymenoptera: Platygastridae) (Turchen et al. 2016).

In our study, no phytotoxic effects on the treated plants were highlighted at all the tested concentrations. EOs due their extremely heterogeneous pool of secondary metabolites may have different impact on plants depending also on the concentration and the kind of formulation. The phytotoxic effect on tomato plants caused by citrus peel EOs was concentration-dependent, and the EO emulsions caused more damage than the PEG EO-nanoparticles formulation (Campolo et al. 2017). The EO adverse effects on plants are considered negative for plant protection from insects but they represent a resource for the development of bio-herbicides. Rolli et al. (2014) screened the phytotoxicity of 25 EOs at pre and post-emergence growth using *S. lycopersicum* and highlighted *Pelargonium capitatum*(L) (Geraniaceae) and *Aniba rosaeodora* Ducke (Lauraceae) EOs eligible as herbicides since these EOs strongly affected both the seed germination as well as plant survival.

In conclusion, the newly developed GEO-based formulation in this research showed promising results in controlling different *T. absoluta* stages with low mortality towards the predator *N. tenuis* and no phytotoxicity on tomato plants. Specifically, the ovicidal and larvicidal activities together with the oviposition deterrence and the lack of phytotoxicity are noteworthy because these different effects can act in parallel synergism in controlling one of the most important tomato pests.

Acknowledgements

This research was supported by the Italian Ministry of Education, University and Research (MIUR) (PRIN project 2015 “BIOPIC”, 2015BABFCF), the EU, Programme IEV de Coopération TransfrontalièreItalie- Tunisie 2014–2020 (Project INTEMAR-IS_ 2.1_ 073 Innovations dans la lute intégrée contre les ravageurs et maladies récemment introduits sur cultures maraîchères, Grant number E64I18002460007 and The University of Catania (University Research Funds—Research Plan 2016/2018).

References

Abbott, W. S. (1925). A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology*, *18*(2), 265-267. https://doi.org/10.1093/jee/18.2.265a

Amagase, H. (2006). Clarifying the Real Bioactive Constituents of Garlic. *The Journal of Nutrition 136* (3). Oxford University Press: 716S–725S. https://doi.org/10.1093/jn/136.3.716S

Amer, S. A. A., & Momen, F. M. (2002). Effect of Some Essential Oils on the Predacious Mite *Amblyseius swirskii* A. H. (Acari: Phytoseiidae). *Acta Phytopathologica et Entomologica Hungarica*, *37* (1–3). Akadémiai Kiadó: 281–86. https://doi.org/10.1556/APhyt.37.2002.1-3.27

Anastassiadou, M., Arena, M., Auteri, D., Brancato, A., Bura, L., Carrasco Cabrera, L.,... & Villamar-Bouza, L. (2020). Peer review of the pesticide risk assessment of the active substance garlic extract. *EFSA Journal*, *18*(6), e06116. https://doi.org/10.2903/j.efsa.2020.6116

Anjali, C.H., Sharma, Y., Mukherjee, A., & Chandrasekaran, N. (2012). Neem Oil (*Azadirachta Indica*) nanoemulsion-a potent larvicidal agent against *Culex quinquefasciatus*. *Pest Management Science*, *68* (2), 158–163. https://doi.org/10.1002/ps.2233

Arnó, J., & Gabarra, R. (2011). Side effects of selected insecticides on the *Tuta absoluta* (Lepidoptera: Gelechiidae) predators *Macrolophus pygmaeus* and *Nesidiocoris tenuis* (Hemiptera: Miridae). *Journal of Pest Science*, *84*(4), 513-520. https://doi.org/10.1007/s10340-011-0384-z

Asadi, M., Rafiee-Dastjerdi, H., Nouri-Ganbalani, G., Naseri, B., & Hassanpour, M. (2018). The effects of plant essential oils on the functional response of *Habrobracon hebetor* Say (Hymenoptera: Braconidae) to its host. *Invertebrate Survival Journal*, *15*(1), 169-182. https://doi.org/10.25431/1824-307x/isj.v15i1.169-182

Athanassiou, C. G., Kavallieratos, N. G., Benelli, G., Losic, D., Usha Rani, P., & Desneux, N. (2018). Nanoparticles for pest control: current status and future perspectives. *Journal of Pest Science, 91*(1), 1–15. https://doi.org/10.1007/s10340-017-0898-0

Biondi, A., Desneux, N., Siscaro, G., & Zappalà, L. (2012). Using organic-certified rather than synthetic pesticides may not be safer for biological control agents: selectivity and side effects of 14 pesticides on the predator *Orius laevigatus*. *Chemosphere*, *87* (7), 803–812. https://doi.org/10.1016/j.chemosphere.2011.12.082

Biondi, A., Guedes, R.N.C., Wan, F.H., & Desneux, N. (2018). Ecology, worldwide spread, and management of the invasive South American tomato pinworm, *Tuta absoluta*: past, present, and future. *Annual Review of Entomology, 63*(1), 239–258. https://doi.org/10.1146/annurev-ento-031616-034933

Campolo, O., Cherif, A., Ricupero, M., Siscaro, G., Grissa-Lebdi, K., Russo, A., … Palmeri, V. (2017). Citrus peel essential oil nanoformulations to control the tomato borer, *Tuta absoluta*: chemical properties and biological activity. *Scientific Reports*, *7,* 103036. https://doi.org/10.1038/s41598-017-13413-0

Campolo, O., Giunti, G., Laigle, M., Michel, T., & Palmeri, V. (2020a). Essential oil-based nano-emulsions: Effect of different surfactants, sonication and plant species on physicochemical characteristics. *Industrial Crops and Products, 157*, 112935. https://doi.org/10.1016/j.indcrop.2020.112935

Campolo, O., Puglisi, I., Barbagallo, R.N., Cherif, A., Ricupero, M., Biondi, A., … Zappalà, L. (2020b). Side effects of two citrus essential oil formulations on a generalist insect predator, plant and soil enzymatic activities. *Chemosphere*, *257*, 127252. https://doi.org/10.1016/j.chemosphere.2020.127252

Campolo, O., Romeo, F.V., Malacrinò, A., Laudani, F., Carpinteri, G., Fabroni, S., … Palmeri, V. (2014). Effects of inert dusts applied alone and in combination with sweet orange essential oil against *Rhyzopertha dominica* (Coleoptera: Bostrichidae) and wheat microbial population. *Industrial Crops and Products*, *61*, 361–369. https://doi.org/10.1016/j.indcrop.2014.07.028

Carvalho, F. P. (2006). Agriculture, pesticides, food security and food safety. *Environmental science & policy*, *9*(7-8), 685-692. https://doi.org/10.1016/j.envsci.2006.08.002

Castilhos, R.V., Grützmacher, A.D., & Coats, J.R. (2018). Acute toxicity and sublethal effects of terpenoids and essential oils on the predator *Chrysoperla externa* (Neuroptera: Chrysopidae). *Neotropical Entomology*, *47*(2), 311–317. https://doi.org/10.1007/s13744-017-0547-6

Cincotta, F., Condurso, C., Tripodi, G., Merlino, M., Prestia, O., Stanton, C., & Verzera, A. (2021). Comparison of lactose free and traditional mozzarella cheese during shelf-life by aroma compounds and sensory analysis. *LWT*, *140*, 110845. https://doi.org/10.1016/j.lwt.2020.110845

Condurso, C., Cincotta, F., Tripodi, G., Merlino, M., & Verzera, A. (2019). Influence of drying technologies on the aroma of Sicilian red garlic. *LWT*, *104*, 180-185. https://doi.org/10.1016/j.lwt.2019.01.026

Desneux, N., Decourtye, A., & Delpuech, J. M. (2007). The sublethal effects of pesticides on beneficial arthropods. *Annual Review of Entomology*, *52*, 81-106. https://doi.org/10.1146/annurev.ento.52.110405.091440

Desneux, N., Han, P., Mansour, R., Arnó, J., Brévault, T., Campos, M. R., ... Biondi, A. (2022). Integrated pest management of *Tuta absoluta*: practical implementations across different world regions. *Journal of Pest Science*, *95*, 17–39. https://doi.org/10.1007/S10340-021-01442-8

Donsì, F., & Ferrari, G. (2016). Essential oil nanoemulsions as antimicrobial agents in food. *Journal of Biotechnology*, *233*, 106–120. https://doi.org/10.1016/J.JBIOTEC.2016.07.005

Galland, C., Glesner, V., & Verheggen, F. (2020). Laboratory and field evaluation of a combination of attractants and repellents to control Drosophila suzukii. *Entomologia Generalis*, 40(3), 263 – 272. DOI: 10.1127/entomologia/2020/1035

González, J.O.W., Laumann, R.A., Da Silveira, S., Moraes, M.C.B., Borges, M., & Ferrero, A.A. (2013). Lethal and sublethal effects of four essential oils on the egg parasitoids *Trissolcus basalis*. *Chemosphere*, *92*(5), 608-615. https://doi.org/10.1016/j.chemosphere.2013.03.066

Goudarzvand Chegini, S., & Abbasipour, H. (2017). Chemical composition and insecticidal effects of the essential oil of cardamom, *Elettaria cardamomum* on the tomato leaf miner, *Tuta absoluta*. *Toxin Reviews*, *36*(1), 12–17. https://doi.org/10.1080/15569543.2016.1250100

Guedes, R. N. C., Roditakis, E., Campos, M. R., Haddi, K., Bielza, P., Siqueira, H. A. A., ... & Nauen, R. (2019). Insecticide resistance in the tomato pinworm *Tuta absoluta*: patterns, spread, mechanisms, management and outlook. *Journal of Pest Science*, *92*(4), 1329-1342. https://doi.org/10.1007/s10340-019-01086-9

Huang, Y., Chen, S. X., & Ho, S. H. (2000). Bioactivities of methyl allyl disulfide and diallyl trisulfide from essential oil of garlic to two species of stored-product pests, *Sitophilus zeamais* (Coleoptera: Curculionidae) and *Tribolium castaneum* (Coleoptera: Tenebrionidae). *Journal of Economic Entomology*, *93*(2), 537-543. https://doi.org/10.1603/0022-0493-93.2.537

Isman, M. B. (2020). Botanical insecticides in the twenty-first century—fulfilling their promise?. *Annual Review of Entomology*, *65*, 233-249. https://doi.org/10.1146/annurev-ento-011019-025010

Jirovetz, L., Jäger, W., Koch, H. P., & Remberg, G. (1992). Investigations of volatile constituents of the essential oil of Egyptian garlic (*Allium sativum* L.) by means of GC-MS and GC-FTIR. *Zeitschrift für Lebensmittel-Untersuchung und Forschung*, *194*(4), 363-365. https://doi.org/10.1007/BF01193221

Kimbaris, A. C., Papachristos, D. P., Michaelakis, A., Martinou, A. F., & Polissiou, M. G. (2010). Toxicity of plant essential oil vapours to aphid pests and their coccinellid predators. *Biocontrol science and technology*, *20*(4), 411-422. https://doi.org/10.1080/09583150903569407

Liu, X. C., Lu, X. N., Liu, Q. Z., & Liu, Z. L. (2014). Evaluation of insecticidal activity of the essential oil of *Allium chinense* G. Don and its major constituents against *Liposcelis bostrychophila* Badonnel. *Journal of Asia-Pacific Entomology*, *17*(4), 853-856. https://doi.org/10.1016/J.ASPEN.2014.08.007

Macías, F. A., Castellano, D., & Molinillo, J. M. (2000). Search for a standard phytotoxic bioassay for allelochemicals. Selection of standard target species. *Journal of agricultural and food chemistry*, *48*(6), 2512-2521. https://doi.org/10.1021/JF9903051

Mansour, R., & Biondi, A. (2021). Releasing natural enemies and applying microbial and botanical pesticides for managing *Tuta absoluta* in the MENA region. *Phytoparasitica*, *49*(2), 179-194. https://doi.org/10.1007/s12600-020-00849-w

Miething, H. (1988). HPLC-analysis of the volatile oil of garlic bulbs. *Phytotherapy Research*, *2*(3), 149-151. https://doi.org/10.1002/ptr.2650020310

Mossa, A.T.H., Afia, S.I., Mohafrash, S.M., & Abou-Awad, B.A. (2018). Formulation and characterization of garlic (*Allium sativum* L.) essential oil nanoemulsion and its acaricidal activity on eriophyid olive mites (Acari: Eriophyidae). *Environmental Science and Pollution Research*, *25*(11), 10526-10537. https://doi.org/10.1007/s11356-017-0752-1

Palermo, D., Giunti, G., Laudani, F., Palmeri, V., & Campolo, O. (2021). Essential oil-based nano-biopesticides: Formulation and bioactivity against the confused flour beetle *Tribolium confusum*. *Sustainability*, *13*(17), 9746. https://doi.org/10.3390/SU13179746

Park, I.K., Choi, K.S., Kim, D.H., Choi, I.H., Kim, L.S., Bak, W.C., ... & Shin, S.C. (2006). Fumigant activity of plant essential oils and components from horseradish (*Armoracia rusticana*), anise (*Pimpinella anisum*) and garlic (*Allium sativum*) oils against *Lycoriella ingenua* (Diptera: Sciaridae). *Pest Management Science*, *62*(8), 723-728. https://doi.org/10.1002/ps.1228

Passos, L.C., Ricupero, M., Gugliuzzo, A., Soares, M.A., Desneux, N., Carvalho, G.A., … & Biondi, A. (2022). Does the dose make the poison? Neurotoxic insecticides impair predator orientation and reproduction even at low concentrations. *Pest Management Science*, *78*(4):1698-1706. https://doi.org/10.1002/ps.6789

Pavela, R., & Benelli, G. (2016). Essential oils as ecofriendly biopesticides? Challenges and constraints. *Trends in plant science*, *21*(12), 1000-1007. https://doi.org/10.1016/j.tplants.2016.10.0053

Pavela, R., Morshedloo, M. R., Mumivand, H., Khorsand, G. J., Karami, A., Maggi, F., ... & Benelli, G. (2020). Phenolic monoterpene-rich essential oils from Apiaceae and Lamiaceae species: insecticidal activity and safety evaluation on non-target earthworms. *Entomologia Generalis*, 421-435. DOI: 10.1127/entomologia/2020/1131

Pavela, R., Pavoni, L., Bonacucina, G., Cespi, M., Cappellacci, L., Petrelli, R., ... & Benelli, G. (2021). Encapsulation of *Carlina acaulis* essential oil and carlina oxide to develop long-lasting mosquito larvicides: Microemulsions versus nanoemulsions. *Journal of Pest Science, 94*(3), 899-915. Doi: 10.1007/s10340-020-01327-2

Rabinowitch, H.D., & Currah, L. (2002). Allium crop science: recent advances. Wallingford, UK: CABI Publishing. https://doi.org/10.1079/9780851995106.0000

Regnault-Roger, C., Vincent, C., & Arnason, J.T. (2012). Essential oils in insect control: low-risk products in a high-stakes world. *Annual Review of Entomology*, *57*(1), 405-424. doi:10.1146/annurev-ento-120710-100554

Ribeiro, N., Camara, C., & Ramos, C. (2016). Toxicity of essential oils of *Piper marginatum* Jacq. against *Tetranychus urticae* Koch and *Neoseiulus californicus* (McGregor). *Chilean journal of agricultural research*, *76*(1), 71-76. https://doi.org/10.4067/S0718-58392016000100010

Rolli, E., Marieschi, M., Maietti, S., Sacchetti, G., & Bruni, R. (2014). Comparative phytotoxicity of 25 essential oils on pre-and post-emergence development of *Solanum lycopersicum* L.: A multivariate approach. *Industrial Crops and Products*, *60*, 280-290. https://doi.org/10.1016/j.indcrop.2014.06.021

Satyal, P., Craft, J.D., Dosoky, N.S., & Setzer, W.N. (2017). The chemical compositions of the volatile oils of garlic (*Allium sativum*) and wild garlic (*Allium vineale*). *Foods*, *6*(8), 63. https://doi.org/10.3390/foods6080063

Sciortino, M., Scurria, A., Lino, C., Pagliaro, M., D'Agostino, F., Tortorici, S., ... & Ciriminna, R. (2021). Silica-microencapsulated orange oil for sustainable pest control. Advanced Sustainable Systems, 5(4), 2000280. https://doi.org/10.1002/adsu.202000280

Soares, M. A., Passos, L. C., Campos, M. R., Collares, L. J., Desneux, N., & Carvalho, G. A. (2019). Side effects of insecticides commonly used against *Tuta absoluta* on the predator *Macrolophus basicornis*. *Journal of Pest Science*, *92*(4), 1447-1456. https://doi.org/10.1007/s10340-019-01099-4

Soares, M. A., Campos, M. R., Passos, L. C., Carvalho, G. A., Haro, M. M., Lavoir, A. V., ... & Desneux, N. (2019b). Botanical insecticide and natural enemies: a potential combination for pest management against *Tuta absoluta*. *Journal of Pest Science*, *92*(4), 1433-1443. https://doi.org/10.1007/s10340-019-01102-y

Tang, F.H., Lenzen, M., McBratney, A., & Maggi, F. (2021). Risk of pesticide pollution at the global scale. *Nature Geoscience*, *14*(4), 206-210. https://doi.org/10.1038/s41561-021-00712-5

Thomson, M., & Ali, M. (2003). Garlic [*Allium sativum*]: a review of its potential use as an anti-cancer agent. *Current cancer drug targets*, *3*(1), 67-81. https://doi.org/10.2174/1568009033333736

Tomé, H.V.V., Cordeiro, E.M.G., Rosado, J.F., & Guedes, R.N.C. (2012). Egg exposure to pyriproxyfen in the tomato leaf miner *Tuta absoluta*: ovicidal activity or behavioural-modulated hatching mortality?. *Annals of Applied Biology*, *160*(1), 35-42. https://doi.org/10.1111/j.1744-7348.2011.00518.x

Turchen, L.M., Piton, L.P., Dall'Oglio, E.L., Butnariu, A.R., & Pereira, M.J.B. (2016). Toxicity of *Piper aduncum* (Piperaceae) essential oil against *Euschistus heros* (F.)(Hemiptera:

Pentatomidae) and non-effect on egg parasitoids. *Neotropical entomology*, *45*(5), 604-611. https://doi.org/10.1007/s13744-016-0409-7

Umpiérrez, M.L., Paullier, J., Porrini, M., Garrido, M., Santos, E., & Rossini, C. (2017). Potential botanical pesticides from Asteraceae essential oils for tomato production: Activity against whiteflies, plants and bees. *Industrial Crops and Products*, *109*, 686-692. https://doi.org/10.1016/j.indcrop.2017.09.025

Vergel, S.J.N., Bustos, R.A., Rodríguez, C.D., & Cantor, R.F. (2011). Laboratory and greenhouse evaluation of the entomopathogenic fungi and garlic–pepper extract on the predatory mites, *Phytoseiulus persimilis* and *Neoseiulus californicus* and their effect on the spider mite *Tetranychus urticae*. *Biological Control*, *57*(2), 143-149. https://doi.org/10.1016/j.biocontrol.2011.02.007

Yang, F.L., Liang, G.W., Xu, Y.J., Lu, Y.Y., & Zeng, L. (2010b). Diatomaceous earth enhances the toxicity of garlic, *Allium sativum*, essential oil against stored-product pests. *Journal of Stored Products Research*, *46*(2), 118-123. https://doi.org/10.1016/j.jspr.2010.01.001

Yang, F.L., Zhu, F., & Lei, C.L. (2010a). Garlic essential oil and its major component as fumigants for controlling *Tribolium castaneum* (Herbst) in chambers filled with stored grain. *Journal of Pest Science*, *83*(3), 311-317. https://doi.org/10.1007/s10340-010-0300-y

Yarou, B.B., Bawin, T., Boullis, A., Heukin, S., Lognay, G., Verheggen, F.J., & Francis, F. (2018). Oviposition deterrent activity of basil plants and their essentials oils against *Tuta absoluta* (Lepidoptera: Gelechiidae). *Environmental Science and Pollution Research*, *25*(30), 29880-29888. https://doi.org/10.1007/s11356-017-9795-6

Zhao, N.N., Zhang, H., Zhang, X.C., Luan, X.B., Zhou, C., Liu, Q.Z., ... & Liu, Z.L. (2013). Evaluation of acute toxicity of essential oil of garlic (*Allium sativum*) and its selected major constituent compounds against overwintering *Cacopsylla chinensis* (Hemiptera: Psyllidae). *Journal of Economic Entomology*, *106*(3), 1349-1354. https://doi.org/10.1603/EC12191

Figure legends

Figure 1. Mean value (± SE) of average size and surface charge trend of the GEO-NE measured during the 16 weeks of survey.

Figure 2. Mean percentages (±SE) of mortality of *Tuta absoluta* eggs sprayed with different GEO-NE application rates. Spinosad-based treated control was sprayed at label rate. Different letters indicate statistical differences among the treatments for $P<0.05$ (Univariate analysis of Variance followed by Duncan post-hoc test).

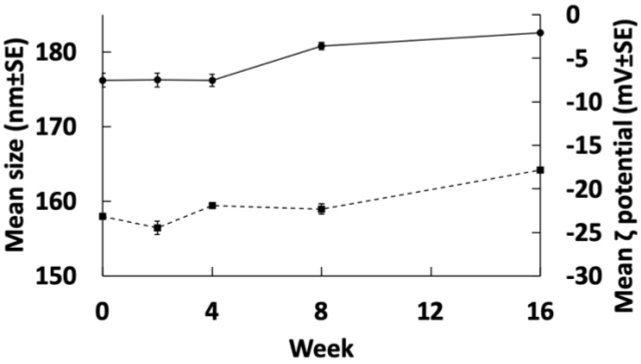
Figure 3. Mean number (±SE) of eggs laid by *Tuta absoluta* females in choice and no-choice tests. Different letters indicate statistical differences between the treatments for $P<0.05$ (choice test: χ^2 goodness of fit; no-choice test: Univariate analysis of Variance followed by Duncan post-hoc test).

Figure 4. Bars show the mean number (±SE) of progeny produced by *Nesidiocoris tenuis* females during 3 d of exposure to 1h and 72h old different concentrations of GEO-NE residues on tomato shoots. Different letters indicate statistical differences between each treatment for $P<0.05$ (Univariate analysis of Variance followed by Duncan post-hoc test). Dashed line indicates the mean number (±SE) of progeny produced in the control.

Table 1. Female, male and total mean percentages (±SE) of mortality of *Nesidiocoris tenuis* adults exposed to 1h and 72h old different concentrations of GEO-NE residues on tomato shoots. Different letters indicate statistical differences between the same treatment for $P<0.05$ (Univariate analysis of Variance followed by Duncan post-hoc test).

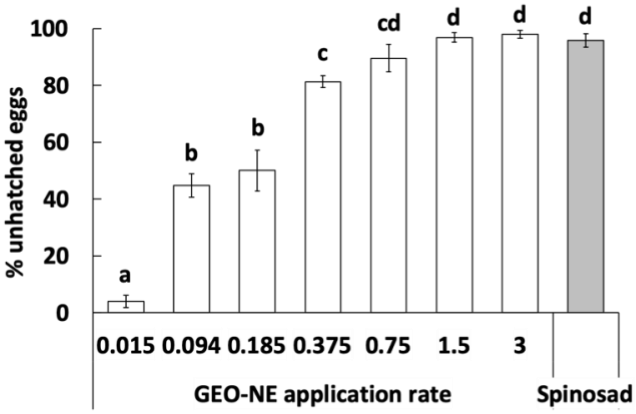
Treatment	**Residual age**	**Male mortality**	**Female mortality**	**Total mortality**
GEO-NE $LC_{50\ eggs}$	1	4.74±2.41a	3.33±2.22a	3.16±1.61a
	72	2.22±1.48a	0±0a	0.43±0.29a
GEO-NE 3%	1	66.84±13.08a	54.17±11.11a	60±11.07a
	72	4.44±3.39b	1.49±1.49b	2.83±1.72b
Indoxacarb	1	91.58±6.43a	77.5±10.15a	84.21±8.04a
	72	73.33±8.64a	68.09±7.27a	70.65±5.39a

Figure 1



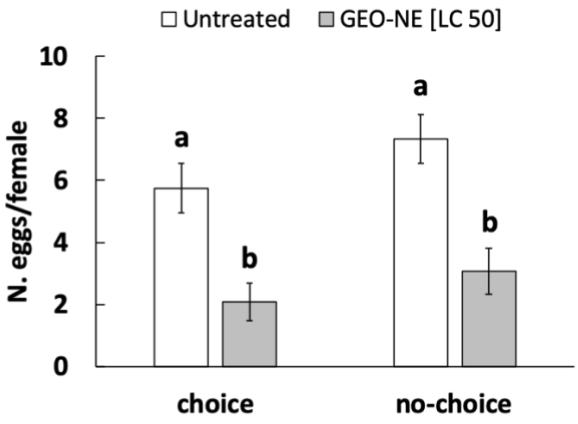
Mean size (nm±SE)
180
170
160
150
0
-5
-10
-15
-20
-25
-30
Mean ζ potential (mV±SE)
0
4
8
12
16
Week

633 **Figure 2**

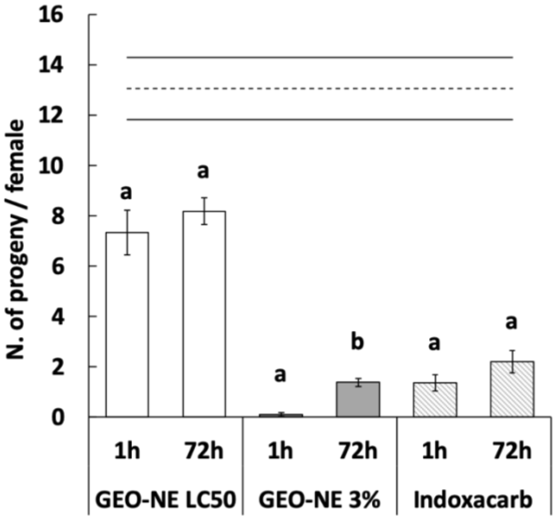


634

Figure 3


Untreated
GEO-NE [LC 50]
N. eggs/female
10
8
6
4
2
0
a
b
a
b
choice
no-choice

637 **Figure 4**



638

Supplementary materials

Table S1. Essential oil composition (Average area %) of garlic (*Allium sativum*)

Compound	LRI[a]	%
Allylmethylsulfide	699	0.62
Dimethyldisulfide	739	0.05
Hexan-3-one	788	* [b]
Hexan-2-one	797	0.02
Hexanal	807	*
4-Methylthiazole	822	0.01
Allylisopropylsulfide	826	0.01
Furfural	832	0.01
1,2-Dithiolane	842	0.03
Diallylsulfide	857	10.69
2,4-Dimethyl thiophene	864	*
Allylpropylsulfide	872	0.03
Allyl (E)-1-propenyl sulfide	890	*
Allylmethyldisulfide	914	2.60
Methylpropyldisulfide	930	0.01
2-Ethoxythiazole	944	0.01
Methyl (E)-1-propenyl disulfide	947	0.11
(E)-2-Hexenal	952	0.02
3H-1,2-Dithiolene	957	0.16
Dimethyltrisulfide	967	0.06
Benzaldehyde	969	0.04
3-(Methylthio)-1-propanol	979	0.01
2-Carboxaldehyde thiophene	1016	*
Allylisopropyldisulfide	1050	0.16
2.5-Dimethyl-4-ethylthiazole	1054	*
1-(Methylthio)-3-pentanone	1067	0.01
Diallyldisulfide	1080	29.66
Allyl (Z)-1-propenyl disulfide	1093	0.22
Allyl (E)-1-propenyl disulfide	1099	0.01
Allylmethyltrisulfide	1138	3.22
4-Methyl-1,2,3-trithiolane	1156	0.95
Methyl (E)-1-propenyl trisulfide	1166	0.01
4,5-Dimethyl-2-propylthiazole	1174	0.01
3-Vinyl-4H-1,2-dithiine	1189	0.03
4H-1,2,3-Trithiine	1200	0.03
Allicin (diallylthiosulfinate)	1208	0.02
2-Vinyl-4H-1,3-dithiine	1215	0.05
4,5-Dimethyl-2-butylthiazole	1226	0.07
Allylisoproyltrisulfide	1266	0.06
4-(Hydroxymethyl)-1,2-dithiepane	1278	0.01

4-Methyl-1,2,5-trithiepane	1285	0.05
Diallyltrisulfide	1303	21.50
Allylpropyltrisulfide	1314	0.12
Allyl (E)-1-propenyl trisulfide	1323	*
(E)-3,5-Diethyl-1,2,4- trithiolane	1342	0.05
Allylmethyltetrasulfide	1357	0.01
5-Methyl-1,2,3,4-tetrathiane	1367	0.06
(Z)-3,5-Diethyl-1,2,4-trithiolane	1374	0.29
2-Heptyl thiophene	1381	1.32
3,6-Dimethyl-1,2,5-trithiepane	1428	0.02
4-Ethyl-2,3,5-trithia-6-octene	1444	0.33
4,6-Dimethyl-1,2,5-trithiepane	1460	0.06
Diallyltetrasulfide	1544	13.19
Allylmethylpentasulfide	1573	0.01
7-Methyl-4,5,8-trithia-1,10-undecadiene	1583	0.33
4-Ethyl-6-methyl-1,2,3,5-tetrathiolane	1588	0.05
6-Methyl-4,5,8-trithia-1,10-undecadiene	1592	1.21
N-propyl-2-Thiopheneacetamide	1622	0.06
4-Methyl-1,2,3,5,6-pentathiepane	1649	0.18
6-Ethyl-4,5,7,8-tetrathianonane	1658	0.39
N-isobutyl-2-Thiopheneacetamide	1663	0.24
Hexathiepane	1680	0.29
Diallylpentasulfide	1755	1.03
Allylmethylhexasulfide	1781	0.47
8-Methyl-4,5,6,9-tetrathia-1,11-dodecadiene	1815	5.54
Diallylhexasulfide	1897	0.22
Allylmethylheptasulfide	1922	0.16
2-Methyl-1,3-benzothiazole	1957	0.36
5-Ethyl-7-pentyl-1,2,3,4,6-pentathiepane	2005	0.32
Cyclooctasulfur	2044	0.08
9-Methyl-4,5,6,7,10-pentathia-1,12-tridecadiene	2051	0.68
8-Methyl-4,5,6,7,10-pentathia-1,12-tridecadiene	2056	0.68

[a] Linear Retention Index calculated on a DB-5ms column; [b]< 0.01%.