Environ Sci Pollut Res (2018) 25:10243–10249
DOI 10.1007/s11356-017-8596-2



# Nano-formulation enhances insecticidal activity of natural pyrethrins against *Aphis gossypii* (Hemiptera: Aphididae) and retains their harmless effect to non-target predators

**Nikos E. Papanikolaou**[1] · **Argyro Kalaitzaki**[2,3] · **Filitsa Karamaouna**[4] · **Antonios Michaelakis**[1] · **Vassiliki Papadimitriou**[2] · **Vassilis Dourtoglou**[5] · **Dimitrios P. Papachristos**[1]

Received: 22 October 2016 / Accepted: 7 February 2017 / Published online: 16 February 2017


**Abstract** The insecticidal activity of a new nano-formulated natural pyrethrin was examined on the cotton aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae), and the predators *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) and *Macrolophus pygmaeus* Rambur (Hemiptera: Miridae), in respect with the nano-scale potential to create more effective and environmentally responsible pesticides. Pyrethrin was nano-formulated in two water-in-oil micro-emulsions based on safe biocompatible materials, i.e., lemon oil terpenes as dispersant, polysorbates as stabilizers, and mixtures of water with glycerol as the dispersed aqueous phase. Laboratory bioassays showed a superior insecticidal effect of the pyrethrin micro-emulsions compared to two commercial suspension concentrates of natural pyrethrins against the aphid. The nano-formulated pyrethrins were harmless, in terms of caused mortality and survival time, to L3 larvae and four-instar nymphs of the predators *C. septempunctata* and *M. pygmaeus*, respectively. We expect that these results can contribute to the application of nano-technology in optimization of pesticide formulation, with further opportunities in the development of effective plant protection products compatible with integrated pest management practices.

**Keywords** Nano-technology · Insecticides · Aphids · Predators · Pyrethrins · Micro-emulsions

Responsible editor: Philippe Garrigues

✉ Dimitrios P. Papachristos
d.papachristos@bpi.gr

[1] Department of Entomology and Agricultural Zoology, Benaki Phytopathological Institute, 8 Stefanou Delta Str, 14561 Kifisia, Greece

[2] Institute of Biology, Medicinal Chemistry and Biotechnology, National Hellenic Research Foundation, Athens, Greece

[3] MTM Research Center, School of Science and Technology, Örebro University, Örebro, Sweden

[4] Department of Pesticides' Control and Phytopharmacy, Benaki Phytopathological Institute, 8 Stefanou Delta Str, 14561 Kifisia, Greece

[5] VIORYL S.A., Afidnes, Greece

## Introduction

Botanical insecticides are key components in integrated pest management (IPM) practices due to their beneficial combination of insecticidal activity and the reduced environmental and health contamination (Isman 2006). Natural pyrethrins are common botanical insecticides, mainly extracted from the capitula of the white pyrethrum daisy, *Chrysanthemum* (*Tanacetum*) *cinerariaefolium* (Asteraceae), and are widely used in agricultural practice (Li et al. 2014). Their competitive lethal activities against several insect pests, as well as the weak development of resistant strains, are, among others, considerable advantages of these insecticides (Hitmi et al. 2000). Although the introduction of synthetic pyrethroids had a negative impact on the commercial use of natural pyrethrins for several years, this trend has been reversed (Hitmi et al. 2000) and evolution of natural pyrethrin insecticides may contribute to their industrial potential.

Nano-technology presents, in turn, an opportunity of developing more effective and low environmental risk insecticides (Rai and Ingle 2012; Das et al. 2014; Mukhopadhyay 2014). Nano-pesticides may cover a wide range of pesticide formulations (Kah et al. 2013) including nano- and micro-emulsions that have been proved valuable tools for use as carriers of natural compounds with potential as biopesticides

(Xu et al. 2010; Ghosh et al. 2013; Kalaitzaki et al. 2015; de Almeida et al. 2015). They may occur as water-in-oil and oil-in-water forms, where the core of the particle is either water or oil, respectively (Sarker 2005). The use of micro- and nano-emulsions as carriers of pesticides decreases the use of organic solvent and increases the dispersity, wettability, and penetration properties of the droplets and may lead to improvement of the biological efficacy of pesticides (Koul et al. 2008; Kah et al. 2013; Kalaitzaki et al. 2015).

Aphids are generally recognized as serious pests in agricultural systems (Blackman and Eastop 2000). They are known to produce a large number of generations throughout the year, as individuals frequently have extremely high growth and developmental rates (Awmack and Leather 2007). The cotton aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae), is a worldwide distributed viviparous polyphagous pest of field and greenhouse crops and a vector of 50 plant viruses (Blackman and Eastop 2000). The host plants of the cotton aphid include cotton, cucurbits, citrus, coffee, cocoa, eggplant, pepper, potato, okra, and many ornamental plants (Blackman and Eastop 2000). Chemical control of aphids with synthetic insecticides in recent decades resulted to insecticide resistance phenomena (Devonshire et al. 1998; IRAC n.d. database) as well as negative effects on natural enemies (e.g., Mizell and Schiffhauer 1990; Kraiss and Cullen 2008; Benelli et al. 2015).

The aphidophagous Coccinellidae and generalist Miridae predators are linked with biological control and integrated pest management strategies of aphids (Obrycki and Kring 1998; Michaud 2012; Zappalá et al. 2013; Papanikolaou and Milonas 2016). The ladybird *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) exists in several habitats in the Palearctic region, where it originates from, but also in the Nearctic region where it has been established (Hodek et al. 2012). *Macrolophus pygmaeus* Rambur (Hemiptera: Miridae) originates from the Mediterranean region. It preys upon several insect pests in greenhouses, such as the whiteflies *Trialeurodes vaporariorum* (Westwood) and *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), the tomato borer *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae), and various aphid species (Perdikis and Lykouressis 2004; Bonato et al. 2006; Desneux et al. 2010; Maselou et al. 2014).

Considering nano-technology as an emerging tool in improvement of pesticides' formulation activity and profile (Wang et al. 2007; Song et al. 2009; Ghosh et al. 2010; Kah et al. 2013), we evaluated the efficacy and possible side effects of new nano-formulations of natural pyrethrins in water-in-oil (W/O) micro-emulsions based exclusively on safe non-toxic materials (Kalaitzaki et al. 2015). For this purpose, laboratory bioassays were conducted in order to evaluate the insecticidal effect of nano-formulated pyrethrins on *A. gossypii*. In addition, as the conservation of aphid natural enemies in agricultural habitats remains an imperative issue, potential adverse effects on the non-target predators *C. septempunctata* and *M. pygmaeus* were also evaluated.

## Materials and methods

### Plant material—insects

Cucumber plants, *Cucumis sativus* L. (F-1 Hybrid, Galileo), were grown at plant growth chambers (25 ± 1 °C, 65 ± 5% RH, and a photoperiod of 16:8 (L:D) h) and were transferred to the aphid colony at the three- to five-leaf stage.

*A. gossypii* was collected from *Cucurbita pepo* L. plants in the Prefecture of Attika (central Greece) in spring 2012. The aphid was reared on cucumber plants, in Plexiglass cages (50 × 30 cm), at 25 ± 1 °C, 65 ± 5% RH, and a photoperiod of 16:8 (L:D) h.

*C. septempunctata* beetles were collected from the area of Arta (northwestern Greece) in spring 2012. A mass culture was established and maintained on the black bean aphid, *Aphis fabae* Scopoli (Hemiptera: Aphididae), originally coming from a stock colony of the Biological Control Laboratory, Benaki Phytopathological Institute. The aphids were provided to the predator on infested potted fava bean, *Vicia faba* L., plants in cylindrical Plexiglass cages (50 × 30 cm), at 25 ± 1 °C, 65 ± 5% RH, and a photoperiod of 16:8 (L:D) h.

*M. pygmaeus* was collected from a greenhouse tomato culture, *Solanum lycopersicum* L., infested by *T. vaporariorum*, in Attika Prefecture (summer 2013) and was reared on tomato plants infested by *B. tabaci* in cylindrical Plexiglass cages (50 × 30 cm), at 25 ± 1 °C, 65 ± 5% RH, and a photoperiod of 16:8 (L:D) h.

### Pyrethrins

A new formulation of natural pyrethrins developed by the Institute of Biology, Medicinal Chemistry, and Biotechnology, National Hellenic Research Foundation, Athens, Greece (Kalaitzaki et al. 2015), containing W/O micro-emulsions that could be diluted in one step with aqueous phase to obtain kinetically stable oil-in-water (O/W) nano-emulsions, was tested for its insecticidal effect compared to the reference product Pyrethro Vioryl 5SC (pyrethrins 5% *w/v*). Two different micro-emulsion systems consisting of lemon oil terpenes/Tween 20/(water/glycerol) (2:1) (System 1) and lemon oil terpenes/Tween 80/(water/glycerol) (2:1) (System 2) were examined. Lemon oil terpenes (97.5%), polyoxyethylene sorbitan monooleate (Tween 80) or polyoxyethylene sorbitan monolaurate (Tween 20), natural pyrethrins (75%), and glycerol were used. The natural pyrethrin solution was a combination of six esters, pyrethrin I (38%), pyrethrin II (35%), cinerin I (7.3%), cinerin II

(11.7%), jasmolin I (4%), and jasmolin II (4%), at a total concentration of 3.1% in the micro-emulsions.

## Insecticidal effect of nano-formulated pyrethrins on the aphid *A. gossypii*

### *A. gossypii*—*laboratory bioassays*

The bioassays concerning the toxicity of the nano-formulated pyrethrins (System 1 and System 2) on *A. gossypii* were conducted using cucumber plants (F-1 Hybrid, Galileo) at one-leaf stage (about 12-cm height and total leaf surface area of approximately 63 $cm^2$). Prior to bioassays, six to eight *A. gossypii* adults were transferred at each plant for 2 days. Two days after their removal, the number of nymphs per plant was counted. The nano-formulated pyrethrins were tested in two doses, 60 and 100 ml experimental formulation/hl water solution (1.86 and 3.1 g a.i./hl, respectively). A blank micro-emulsion identical to the nano-formulated pyrethrin without the pyrethrin was also tested. As chemical control, two commercial formulations of natural pyrethrin were used, Parapin 5SC (pyrethrins 5% *w*/*v*) and Pyrethro Vioryl 5SC (pyrethrins 5% *w*/*v*), at the recommended dose (3.0 g a.i./hl) against *A. gossypii* in citrus (not registered use in cucumber). The bioassays were conducted using a Potter Precision Laboratory Spray Tower (by Burkard Scientific Ltd., UK). The spray volume was 5 ml. Thereafter, the plants were kept individually at 25 ± 1 °C, 65 ± 5% RH, and a photoperiod of 16:8 (L:D). One, 3, and 7 days after the application, the number of alive aphids was counted in each plant. There were 17 replicates in each treatment.

## Effect of nano-formulated pyrethrin on non-target predators—laboratory bioassays

The effect of the nano-formulated pyrethrin on the ladybird *C. septempuctata* and the myrid *M. pygmaeus* was examined only for System 2 due to unavailability of the appropriate amounts of System 1 required for bioassays.

### *Coccinella septempuctata*

The bioassays were carried out according the protocol of Schmuck et al. (2000), i.e., by exposing larvae to dry residues. For this purpose, 4–6-day-old larvae were placed individually in glass Petri dishes (*d* = 11 cm), which had been previously sprayed with the treatment solutions and left to dry. The arena of the predator was defined by placing a glass cylinder (*d* = 5 cm, *h* = 4 cm) in the center of each Petri dish. In order to prevent larvae from climbing on the walls of the cylinders, the inner surface of the cylinders was sprayed with Teflon. To avoid vapor concentration of the treatment solutions and to allow ventilation, lids of plastic Petri dishes bearing openings (5 cm) covered with organdie were used as tops of the cylinders. Larvae of *C. septempuctata* were fed ad libitum with nymphs and adults of the aphid *A. fabae* until death or adulthood, at 25 ± 1 °C, 65 ± 5% RH, and a photoperiod of 16:8 (L:D). The nano-formulated pyrethrin (System 2) was tested in four doses, 1.86, 3.1, 6.2, and 12.4 g a.i./hl, where the registered natural pyrethrin Pyrethro Vioryl 5SC was tested at the recommended dose (3.0 g a.i./hl); the pyrethroid insecticide Decis 2.5 EC was tested at 1.25 g a.i./hl, which is the recommended dose for the control of aphids in protected cucumber; and a blank micro-emulsion identical to the nano-formulated pyrethrin without the pyrethrin was also tested. The spray volume was 200 μl. There were 30–36 replicates in each treatment. Daily observations of the living individuals were made until adult emergence.

### *M. pygmaeus*

The same protocol (Schmuck et al. 2000) was used for the bioassays concerning *M. pygmaeus*. Four-instar nymphs of the predator were used and provided daily eggs of *Ephestia kuehniella* and *Artemia* sp. in abundance until death or adulthood. The nano-formulated pyrethrin (System 2) was tested in three doses, 3.1, 6.2, and 12.4 g a.i./hl, where the registered natural pyrethrin Pyrethro Vioryl 5SC was tested at the dose of 3.0 g a.i./hl, the pyrethroid insecticide Decis 2.5 EC was tested at 1.25 g a.i./hl, and a blank micro-emulsion identical to the nano-formulated pyrethrin without the pyrethrin was also tested. The spray volume was 200 μl and 24–36 replications were conducted for each treatment. Daily observations of the living individuals were made until adult emergence.

## Statistical analysis

Data concerning the number of alive aphids of *A. gossypii* were transformed using square root transformation and submitted to one-way analysis of variance (ANOVA), followed by Holm-Sidak test to separate the means.

Efficacy percentage was estimated by the following formula:

$$E = \left(1 - \frac{T_a}{C_a} \times \frac{C_b}{T_b}\right) \times 100$$

where $E$ denotes the efficacy percentage, $T_b$ the aphid population before chemical application, $T_a$ the aphid population after chemical application, $C_b$ the aphid population at the control before the application, and $C_\alpha$ the aphid population at the control after the application.

Concerning the bioassays on predators, the Kaplan-Meier method was used to estimate the survival curves of *C. septempunctata* and *M. pygmaeus*. In addition, the Kaplan-Meier estimate was used to derive the mean survival times and their 95% confidence intervals. All analyses were done using SigmaPlot (Systat Software, 2008).

## Results

The mean aphid densities (survival), after application with the nano-formulations of pyrethrins, were significantly lower compared to the commercial products of pyrethrins (Pyrethro Vioryl, 5SC, Parapin 5SC), especially 1–3 days after application, indicating a 20–40% increase of efficacy, depending on the dose (Table 1). Furthermore, the nano-formulations of pyrethrins exhibited a significantly higher insecticidal effect at a dose which was 62% of the registered dose for *Aphis* spp. of the reference products (1.86 vs 3.0 g a.s. gr/hl) (Table 1). Efficacy of the tested nano-emulsions in the early assessments was 81–83% at 62% of the recommended dose of the reference emulsions, which gave 55–61% efficacy. Effectiveness of nano-emulsions increased significantly to 94–95% at a similar rate (3.0 g a.s. gr/hl) with the commercial products.

The results were comparable 1–7 days after application, but the efficacy of the higher dose was more persistent compared to the lower dose; the efficacy of the nano-emulsions at 1.86 g a.s./hl was 77–78 vs 52–63% for the commercial formulations with no significant difference for Parapin 5SC, and the efficacy of the nano-emulsions at full dose (3.1 g/hl) was 89–91%, which was significantly higher than the commercial emulsions (Table 1). In addition, the blank nano-formulation did not show an insecticidal effect, since the aphid survival did not differ significantly to the one of the control (Table 1).

The mortality of L3 larvae of *C. septempunctata*, exposed to dry residues of the nano-formulated pyrethrin, was 12.5% at 12.4 g a.i./hl. Hence, the concentration which is approximately four times the recommended dose for *A. gossypii* is lower than the $LC_{50}$ for this predator. The mortality of the immatures at the tested pyrethrin nano-emulsion at all applied doses did not differ from the control (22.2%) or the blank formulation (11.1%), and this was also the case for the Pyrethro Vioryl 5SC (13.3%) at the registered dose for *A. gossypii*. The mortality of the predator was significantly higher at the pyrethroid insecticide Decis 2.5 EC (88.9%) compared to the natural pyrethrins (Table 2 and Fig. 1).

The four-instar nymphs of *M. pygmaeus* showed also low mortality (9.4% at 12.4 g a.i./hl) when exposed to dry residues of the nano-formulated pyrethrin (Table 3 and Fig. 2). The mortality at all the applied doses did not differ from the control (8%), the blank formulation (8.3%), and Pyrethro Vioryl 5SC (10.3%). Moreover, Decis 2.5 EC caused significantly higher mortality (33.3%) to *M. pygmaeus* four-instar nymphs, compared to all the above treatments.

The survival curves of the predators are presented in Figs. 1 and 2, showing also that the average survival time of the immatures until adulthood, after exposure to dry residues of the nano-emulsion at all doses, did not differ from Pyrethro Vioryl 5SC, the control (water), and the blank nano-formulation. The larvae of *C. septempunctata* lived significantly less when exposed to the pyrethroid Decis 2.5 EC (Table 2 and Fig. 1).

## Discussion

Our study indicated that nano-formulation can improve the insecticidal activity of natural pyrethrins against the aphid *A. gossypii*. The nano-formulation seems to be able to enhance substantially (several fold increase) the efficacy of natural

**Table 1** Number of alive aphids of *Aphis gossypii* (mean ± SE) on cucumber plants 1, 3, and 7 days after application with nano-formulated natural pyrethrins (System 1 and System 2)

| Treatment | Mean number of alive aphids | | | |
|---|---|---|---|---|
| | Before treatment | Days after the treatment | | |
| | | 1 | 3 | 7 |
| System 1 (3.1 g a.i./hl) | 26.9 ± 2.5 a | 1.3 ± 0.3 d (95.01%) | 2.3 ± 0.6 d (93.67%) | 14.2 ± 3.7 e (90.68%) |
| System 2 (3.1 g a.i./hl) | 29.2 ± 2.8 a | 2.5 ± 0.8 cd (91.16%) | 3.9 ± 1.1 cd (95.02) | 18.2 ± 4.1 de (89.00%) |
| System 1 (1.86 g a.i./hl) | 28.6 ± 1.9 a | 4.6 ± 0.9 c (83.40%) | 6.7 ± 1.1 c (82.66%) | 36.2 ± 6.5 cd (77.66%) |
| System 2 (1.86 g a.i./hl) | 30.3 ± 3.3 a | 5.1 ± 1.0 c (82.63%) | 7.6 ± 1.5 c (81.44%) | 39.3 ± 8.1 cd (77.11%) |
| Parapin 5SC (3.0 g a.i./hl) | 33.6 ± 3.1 a | 13.9 ± 2.4 b (57.30%) | 17.6 ± 3.0 b (61.23%) | 69.9 ± 10.6 bc (63.29%) |
| Pyrethro Vioryl 5SC (3.0 g a.i./hl) | 32.7 ± 2.9 a | 14.2 ± 1.7 b (55.18%) | 18.0 ± 2.5 b (59.26%) | 89.1 ± 12.5 ab (51.92%) |
| Control (water) | 22.5 ± 1.3 a | 21.8 ± 1.2 a | 30.4 ± 2.3 a | 127.5 ± 15.1 a |
| Blank nano-formulation | 32.7 ± 2.0 a | 31.0 ± 2.5 a | 36.0 ± 3.3 a | 131.7 ± 16.2 a |

Numbers in the parentheses denotes the efficacy. In each column, means followed by the same letter are not statisticaly different Holm-Sidak test ($P \geq 0.05$)

**Table 2** Mean mortality and survival time (±SE, 95% confidence intervals) of the aphidophagous predator *Coccinella septempunctata* after exposure to dry residues of nano-formulated pyrethrin (System 2) at L3 stage

| Treatment | Mortality (%) | Survival time (days) | |
|---|---|---|---|
| | | Mean | 95% CI |
| System 2 (1.86 g a.i./hl) | 19.4 a | 9.8 ± 0.5 | 8.7–10.8 |
| System 2 (3.1 g a.i./hl) | 11.1 a | 11.3 ± 0.4 | 10.5–12.1 |
| System 2 (6.2 g a.i./hl) | 12.9 a | 11.1 ± 0.5 | 10.0–12.1 |
| System 2 (12.4 g a.i./hl) | 12.5 a | 11.3 ± 0.4 | 10.6–12.1 |
| Pyrethro Vioryl 5 SC (3.0 g a.i./hl) | 13.3 a | 10.8 ± 0.6 | 9.6–12.1 |
| Blank nano-formulation | 11.1 a | 10.0 ± 0.5 | 8.9–11.1 |
| Control (water) | 22.2 a | 11.2 ± 0.7 | 9.8–12.6 |
| Decis 2.5 EC (1.25 g a.i./hl) | 88.9 b | 2.3 ± 0.4 | 1.5–3.2 |

**Table 3** Mean mortality and survival time (±SE) of the generalist predator *Macrolophus pygmaeus* after exposure to dry residues of a nano-formulated natural pyrethrin (System 2) as four-instar nymphs

| Treatment | Mortality (%) | Survival time (days) | |
|---|---|---|---|
| | | Mean | 95% CI |
| System 2 (3.1 g a.i./hl) | 4.0 a | 13.5 ± – | – |
| System 2 (6.2 g a.i./hl) | 7.7 a | 12.2 ± 0.7 | 10.7–13.7 |
| System 2 (12.4 g a.i./hl) | 9.4 a | 11.9 ± 0.7 | 10.6–13.3 |
| Pyrethro Vioryl 5 SC (3.0 g a.i./hl) | 10.3 a | 13.0 ± 0.7 | 11.6–14.3 |
| Blank nano-formulation | 8.3 a | 14.9 ± 1.1 | 12.8–17.0 |
| Control (water) | 8.0 a | 12.9 ± 1.0 | 11.0–14.9 |
| Decis 2.5 EC (1.25 g a.i./hl) | 33.3 b | 11.2 ± 0.9 | 9.4–13.1 |

pyrethrins for the control of the aphid *A. gossypii*, succeeding good efficacy at lower doses than the present registered formulations or achieving a very good persisting effect at similar dose. Many aspects may account for enhanced efficacy of nano-formulated pyrethrins such as the improved dispersion, deposition, and leaf adhesion of droplets of the spray solution on leaves surface, which results in increase of insecticide bioavailability to target insect. Moreover, nano-encapsulation of water-insoluble pyrethrins in stable nano-emulsions probably enhances the pesticidal life and enables easier penetration due to the increased interfacial area of the system (Kalaitzaki et al. 2015). Further investigation should be conducted in semi-field and greenhouse or field conditions, where other parameters can affect the efficacy of the nano-emulsions such as the exposure to sunlight, the temperature, and the pest pressure to determine the minimum effective dose and develop the efficacy standards for a commercial formulation.

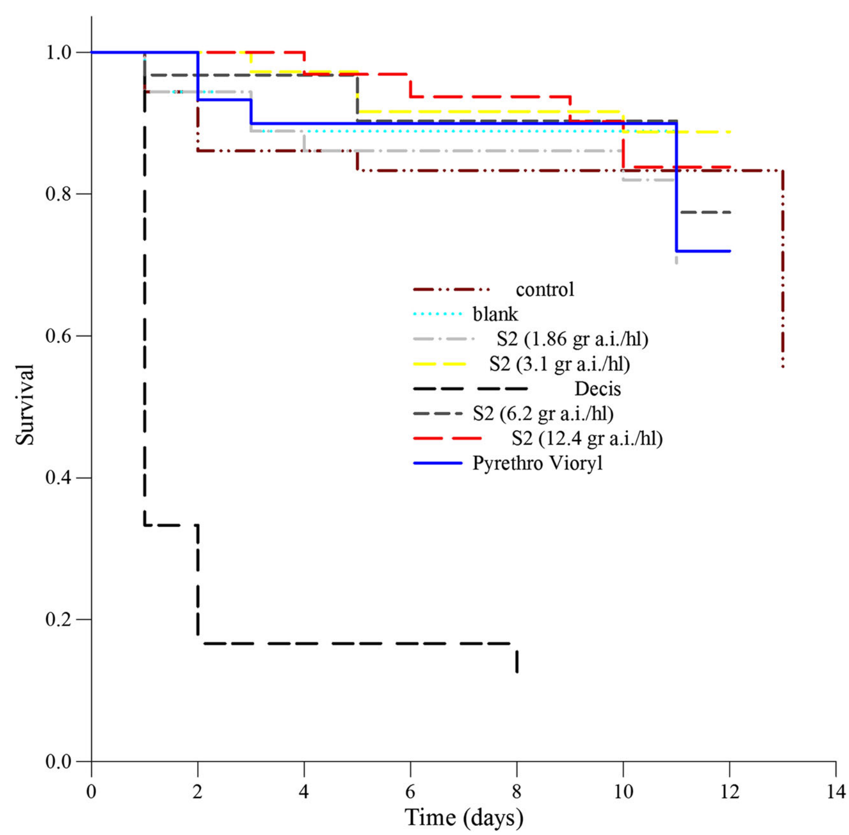
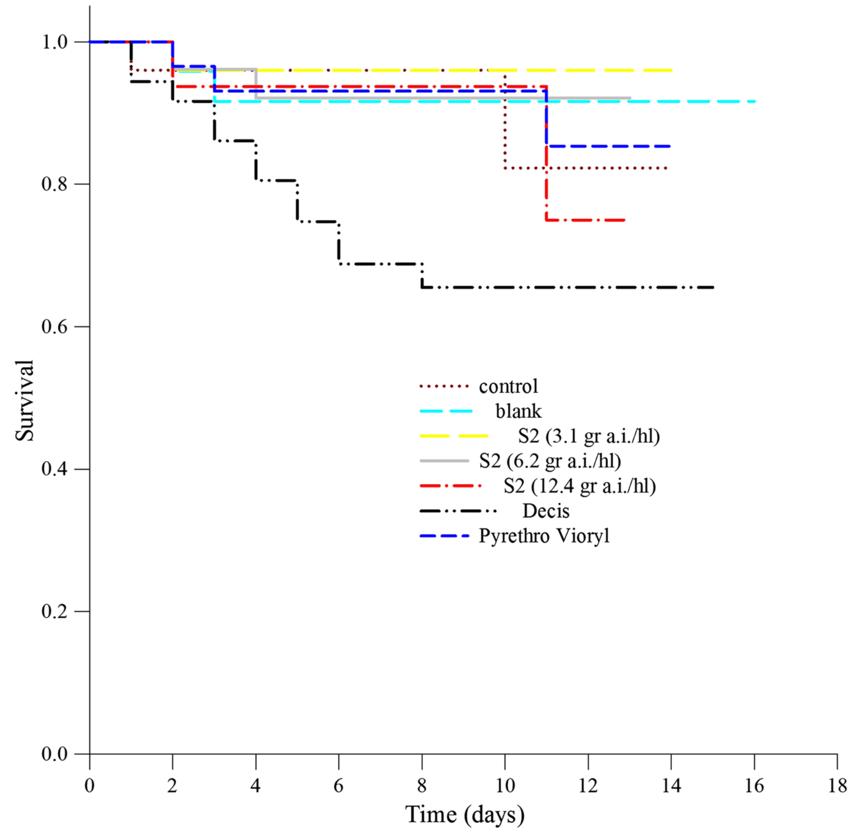



**Fig. 1** Survival curves of immature stages of *Coccinella septempunctata* after exposure to dry residues of nano-formulated pyrethrin (System 2) at L3 stage

**Fig. 2** Survival curves of immature stages of *Macrolophus pygmaeus* after exposure to dry residues of a nano-formulated natural pyrethrin (System 2) as four-instar nymphs




The study also revealed low toxicity of both nano-formulation and commercial formulation of pyrethrins on the non-target predators *C. septempunctata* and *M. pygmaeus* (based on caused mortality) and that the nano-formulation did not increase the effect of pyrethrins on these organisms. In general, pyrethroids—although more effective insecticides compared to pyrethrins—are categorized as harmful on aphid natural enemies, in contrast to harmless or moderate effects of pyrethrins (Desneux et al. 2007; Kraiss and Cullen 2008). Possible adverse effects of the tested nano-dispersions on other aphid biological control agents such as the indicator aphid parasitoid for pesticides' toxicity to beneficial arthropods, *Aphidius rhopalosiphi*, and other *Hymenoptera* parasitoids should be examined to build the toxicity profile of the new nano-emulsion of pyrethrins. However, considering the present results on these main aphid predators, *C. septempuctata* and *M. pygmaeus*, and the general profile of natural pyrethrins, we expect the nano-emulsions to keep the advantage over pyrethroids. Differences in the susceptibility of target insect species and non-target beneficial insects to pyrethrins or pyrethroids have been attributed to differences in the production of the enzyme glutathione transferase, which is capable of metabolizing pyrethrins (Cho et al. 1997; Cho et al. 2002; Desneux et al. 2007).

The increasing insecticidal activity of nano-formulated pyrethrins in combination with the absence of adverse effects on non-target aphid predators make them compatible plant protection products in organic farming and IPM strategies in several crops. Furthermore, the results add further proof to the application of nano-technology in optimization of insecticide formulation for the development of efficacious and reduced environmental risk plant protection products.

**Acknowledgements** The study was funded by the Greek Secretary of Research and Technology within the frame of the common research project "Cooperation," 09-ΣΥΝ-42-699. Authors would like to thank Maria Samara (Laboratory of Efficacy Evaluation of Pesticides, Benaki Phytopathological Institute) for her technical assistance.

## References

Awmack CS, Leather SR (2007) Growth and development. In van Emden HF, Harrington R (eds) Aphids as crop pests. CAB International, pp 135–151

Benelli M, Leather SR, Francati S, Marchetti E, Dindo ML (2015) Effect of two temperatures on biological traits and susceptibility to a pyrethroid insecticide in an exotic and native coccinellid species. Bull Insect 68:23–29

Blackman RL, Eastop VF (2000) Aphids on the world's crops. An identification and information guide, Second edn. John Wiley & Sons, Chinchester

Bonato O, Couton L, Fargues J (2006) Feeding preference of *Macrolophus caliginosus* (Heteroptera: Miridae) on *Bemisia tabaci* and *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae). J Econ Entomol 99:1143–1151

Cho JR, Hong KJ, Yoo JK, Bang JR, Lee JO (1997) Comparative toxicity of selected insecticides to *Aphis citricola*, *Myzus malisuctus* (Homoptera: Aphididae), and the predator *Harmonia axyridis* (Coleoptera: Coccinellidae). J Econ Entomol 90:11–14

Cho JR, Kim J, Kim HS, Yoo JK (2002) Some biochemical evidence on the selective insecticide toxicity between the two aphids, *Aphis citricola* and *Myzus malisuctus* (Homoptera: Aphididae), and their predator, *Harmonia axyridis* (Coleoptera: Coccinellidae). J Asia Pac Entomol 5:49–53

Das RK, Sarma SJ, Brar SK, Verma M (2014) Nanoformulation of insecticides—novel products. J Biofertil Biopestici 5(1):e120. doi:10.4172/2155-6202.1000e120

de Almeida VR, Giongo JL, Bolzan LP, Côrrea MS, Fausto VP, dos Santos Alves CF, Lopes LQS, Boligon AA, Athayde ML, Moreira AP, Brandelli A, Raffin RP, Santos RCV, Brandelli A (2015) Antimicrobial activity of nanostructured Amazonian oils against *Paenibacillus* species and their toxicity on larvae and adult worker bees. J Asia Pac Entomol 18:205–210

Desneux N, Decourtye A, Delpuech J-M (2007) The sublethal effects of pesticides on beneficial arthropods. Annu Rev Entomol 52:81–106

Desneux N, Wajnberg W, Wyckhuys KAG, Burgio G, Arpaia S, Narváez-Vasquez CA, González-Cabrera J, Ruescas DC, Tabone E, Frandon J, Pizzol J, Poncet C, Cabello T, Urbaneza A (2010) Biological invasion of European tomato crops by *Tuta absoluta*: ecology, geographic expansion and prospects for biological control. J Pest Sci 83: 197–215

Devonshire AL, Field LM, Foster SP, Moores GD, Williamson MS, Blackman RL (1998) The evolution of insecticide resistance in the peach-potato aphid, *Myzus persicae*. Phil Trans R Soc Lond B 353: 1677–1684

Ghosh S, Bhowmick DN, Pratap AP (2010) Application of neem and Karanjia oils as natural pesticide microemulsion systems. Tens Surf Deterg 47:369–375

Ghosh V, Saranya S, Mukherjee A, Chandrasekaran N (2013) Cinnamon oil nanoemulsion formulation by ultrasonic emulsification: investigation of its bactericidal activity. J Nanosci Nanotechnol 13:114–122

Hitmi A, Coudret A, Barthomeuf C (2000) The production of pyrethrins by plant cell and tissue cultures of *Chrysanthemum cinerariaefolium* and *Tagetes* species. Crit Rev Biochem Mol 35:317–337

Hodek I, van Emden HF, Honěk A (2012) Ecology and behaviour of the ladybird beetles (*Coccinellidae*). Wiley-Blackwell

IRAC (nd) database <http://www.irac-online.org/>

Isman MB (2006) Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. Annu Rev Entomol 51:45–66

Kah M, Beulke S, Tiede K, Hofmann T (2013) Nanopesticides: state of knowledge, environmental fate, and exposure modelling. Crit Rev Env Sci Tec 43:1823–1867

Kalaitzaki A, Papanikolaou NE, Karamaouna F, Dourtoglou V, Xenakis A, Papadimitriou V (2015) Biocompatible colloidal dispersions as potential formulations of natural pyrethrins: a structural and efficacy study. Langmuir 31(21):5722–5730. doi:10.1021/acs.langmuir.5b00246

Koul O, Walia S, Dhaliwal GS (2008) Essential oils as green pesticides: potential and constraints. Biopestic Int 4:63–84

Kraiss H, Cullen EM (2008) Efficacy and nontarget effects of reduced-risk insecticides on *Aphis glycines* (Hemiptera: Aphididae) and its biological control agent *Harmonia axyridis* (Coleoptera: Coccinellidae). J Econ Entomol 101:391–398

Li J, Jongsma MA, Wang CY (2014) Comparative analysis of pyrethrin content by mass selection, family selection and polycross in pyrethrum [*Tanacetum cinerariifolium* (Trevir.) Sch.Bip.] populations. Ind Crop Prod 53:268–273

Maselou DA, Perdikis DC, Sabelis MW, Fantinou AA (2014) Use of plant resources by an omnivorous predator and the concequences for effective predation. Biol Control 79:92–100

Michaud JP (2012) Coccinellids in biological control. In Hodek I, van Emden HF, Honěk A (eds) Ecology and behaviour of the ladybird beetles (Coccinellidae). Wiley-Blackwell, pp 488–519

Mizell RF, Schiffhauer DE (1990) Effects of pesticides on pecan aphid predators *Chrysoperla rufilabris* (Neuroptera: Chrysopidae), *Hippodamia convergens*, *Cycloneda sanguinea* (L.), *Olla v-nigrum* (Coleoptera: Coccinellidae), and *Aphelinus perpallidus* (hymenoptera: Encyrtidae). J Econ Entomol 83:1806–1812

Mukhopadhyay SS (2014) Nanotechnology in agriculture: prospects and constraints. Nanotechnol Sci Appl 7:63–71

Obrycki JJ, Kring TJ (1998) Predaceous coccinellids in biological control. Annu Rev Entomol 43:295–321

Papanikolaou NE, Milonas PG (2016) Aphidophagous ladybird beetles as biological control agents. In Travlos, IS, Bilalis D, Chachalis D (eds) Weed and Pest Control: Molecular Biology, Practices and Environmental Impact. Nova Science Publishers Inc, pp 143–156

Perdikis DC, Lykouressis DP (2004) *Myzus persicae* (Homoptera: Aphididae) as a suitable prey for *Macrolophus pygmaeus* (Hemiptera: Miridae) population increase on pepper plant. Environ Entomol 33:499–505

Rai M, Ingle A (2012) Role of nanotechnology in agriculture with special reference to management of insect pests. Appl Microbiol Biotechnol 94:287–293

Sarker DK (2005) Engineering of nanoemulsions for drug delivery. Curr Drug Deliv 2:297–310

Schmuck R, Candolfi MP, Kleiner R, Mead-Briggs M, Moll M, Kemmeter F, Jans D, Waltersdorfer A, Wilhelmy H (2000) A laboratory test system for assessing effects of plant protection products on the plant dwelling insect *Coccinella septempunctata* L. (Coleoptera: Coccinellidae). In Candolfi MP, Blumel S, Forster R (eds.), Guidelines to evaluate side-effects of plant protection products to non-target arthropods. IOBC, BART and EPPO Joint Initiative, pp. 45–56

Song S, Liu X, Jiang J, Qian Y, Zhang N, Wu Q (2009) Stability of triazophos in self-nanoemulsifying pesticide delivery system. Colloids Surf A Physicochem Eng Asp 350:57–62

Wang L, Li X, Zhang G, Dong J, Eastoe J (2007) Oil-in-water nanoemulsions for pesticide formulations. J. Colloid Interface Sci 314:230–235

Xu J, Fan QJ, Yin ZQ et al (2010) The preparation of neem oil microemulsion (*Azadirachta indica*) and the comparison of acaricidal time between neem oil microemulsion and other formulations in vitro. Vet Parasitol 169:399–403

Zappalá L, Biondi A, Alma A et al (2013) Natural enemies of the south American moth, *Tuta absoluta*, in Europe, North Africa and Middle East, and their potential use in pest control strategies. J Pest Sci 86: 635–647