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Evaluation of the effect of calcium and nano calcium on *Agonoscena pistaciae* (Hem.: Aphalaridae) in pistachio orchards

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Abstract
Calcium (Ca) is necessary for the construction of cell walls and a wide range of physiological activities in plants. In this study Ca nanoparticles were produced from a solution containing Ca Sulfate by solvothermal method and were investigated in relation to varying fertilization of macroscales and nanoparticles of Ca on one of the important pistachios pests, the common pistachio psylla, *Agonoscena pistaciae*, fed on pistachio saplings of Ahmadaghaei rootstock (20-year-old) in pistachio orchard. The population density of *A. pistaciae* was tested using complete randomized blocks with three replications and four treatments: Ca, nano-Ca, pesticide (acetamiprid), and control (distilled water). The results revealed that the influence of feeding solution on nymph and egg populations differed significantly at the % 1 level, with acetamiprid having the highest measure of control on eggs and Ca having the lowest. In addition, acetamiprid boosted nymph abundance whereas Ca lowered it.

Keywords: Common pistachio psylla, calcium, fertilization, macroscales, nanoparticles

Introduction
Nanoparticle research is emerging as one of the key techniques of the twenty-first century [1]. Nanotechnology is the study and high technology of nanoscale science (1 to 100 nm), is a promising branch of study that has opened up a wide range of prospects in the last decade and raises optimism for the future to tackle agricultural challenges [1, 2]. Nanoparticles are a new generation of environmentally friendly construction technologies with the potential to provide a cost-effective solution to some of the most complex environmental remediation problems, including insecticides and fertilizer buildup [2, 3].
The common pistachio psylla (CPP), *Agonoscena pistaciae* Burckhardt and Lauterer (Hem.: Aphalaridae) is the main pest of pistachio orchards in Iran because of its vast distribution [4, 5]. It also is detected in different pistachio-growing regions near Iran's borders, such as Armenia and Turkey, as well as Mediterranean countries like Greece and Syria [6, 7]. Given the increasing resistance of the CPP to standard insecticides, the subsequent and repeated invasion of other pests after spraying, as well as the pest`s escalation as a response to recompense for population loss after spraying, chemical control appears to be an ineffective strategy for managing the CPP`s population [8]. Consequently, a non-toxic strategy to protect pistachios trees against this pest must be created. Despite the fact that the nature and scope of the evolutionary link between plants and herbivores are currently being disputed [9, 10]. Calcium is necessary for the construction of cell walls and other physiological functions in trees [11]. Researches showed that calcium decreased the abundance of pests [12, 13] when decreasing the size of materials to the nanometer-scale caused a prominent mutation in their properties [14].
In this investigation, we synthesized nanoparticles of calcium by solvothermal method, and the effect of macroscales and nanoparticles fertilization of calcium was investigated on the population density of *A. pistaciae*.

Materials and Methods
Synthesis of CaO nanoparticles
Sodium hydroxide solution (4 M, 10 mL) was added to a solution of $CaSo_4$ (2 mmol) in EtOH/H_xO solvent (25 mL).

To investigate the role of surfactants on the size and morphology of nanoparticles, we used 0.5g of polyethylene glycol (PEG) in the reactions with optimized conditions. The obtained mixtures were sonicated for 150 min with ultrasound powers followed by centrifugation and separation of the solid and liquid phases.

Study site

This investigation was performed in Ahmadaghaei rootstock orchards as the most common pistachio trees in Rafsanjan, Iran. This study was done on the same trees with similar ages and horticultural operations. The complete randomized blocks with three replications were selected for testing four treatments: Ca, nano-Ca, pesticide (acetamiprid), and control (distilled water) against the CPP fed on the same age trees, Ahmadaghaei rootstock (25-year-old). In this study the calcium prepared by Merck and Bayer, Germany was used and the time and consideration of calcium and pesticide spraying were selected according to the common time of spraying by farmers and recommended time by the Pistachio Research Center of Iran.

Sampling

Regarding Rouhani *et al*. [7], 72 hours after spraying, sampling was started and repeated every three days thereafter. The number of nymphs on top of and under each leaf was counted, and averages were determined for the density of each developmental stage per treatment and month, as well as their replicates, to arrive at averages per treatment and block.

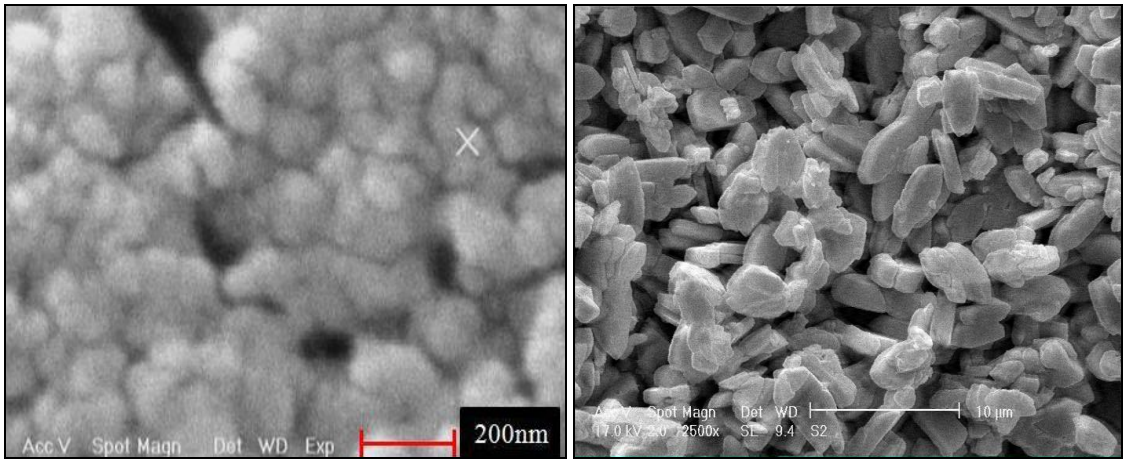
Statistical analysis

The synthesized nanoparticles were characterized by a scanning electron microscope (SEM) (Philips XL 30) for determining their morphology and size. SPSS 22 software was used for data analysis with one-way analysis of variance (ANOVA) and Excel 2019 software was used to create the charts.

Results

Structural study of nanoparticles

Scanning Electron Microscopy (SEM) was used to examine the size of synthesized nanoparticles. The initial form of the Ca nanoparticles is essentially spherical, having a diameter of 44 nm, as shown in Figure 1.



A B

Fig 1: The SEM images of synthesized calcium nanoparticles (a) and Calcium macroscales (b)

Effect of fertilization on *Agonoscena pistaciae*

According to the results, the effect of treatments had a significant difference at %1 level on the egg ($F_{3,152}$=23.3, P=0.00) and nymph instars ($F_{3,152}$=15.46, P=0.00). The grouping and the similarity degree were calculated at a 5% level. (Table 1).

Table 1: Means (± SE) effect of nano-calcium on the population of eggs and total nymphal of *A. pistaciae*

Treatment	Egg	Nymphal periods
Nano-Ca	17.56 ± 1.18^{b}	9.53 ± 0.88^{c}
Ca	20.31 ± 0.87^{a}	14.79 ± 1.4^{b}
Acetamiprid	13.88 ± 0.83^{c}	21.88 ± 0.99^{a}
Control	20.15 ± 1.15^{a}	20.15 ± 1.15^{a}

The similar characters in column indicate the lack of significant difference at 5% level

As indicated in Figure 2, acetamiprid had the highest measure of control on eggs, while nano-Ca had the lowest, and there was no significant difference between the treatments and the control.

In comparison to the control, acetamiprid greatly increased the population of nymphs, while Ca significantly decreased the number of nymphs (Figure 3). These findings revealed that nano-Ca reduced the nymph population more than macroscales of Ca.

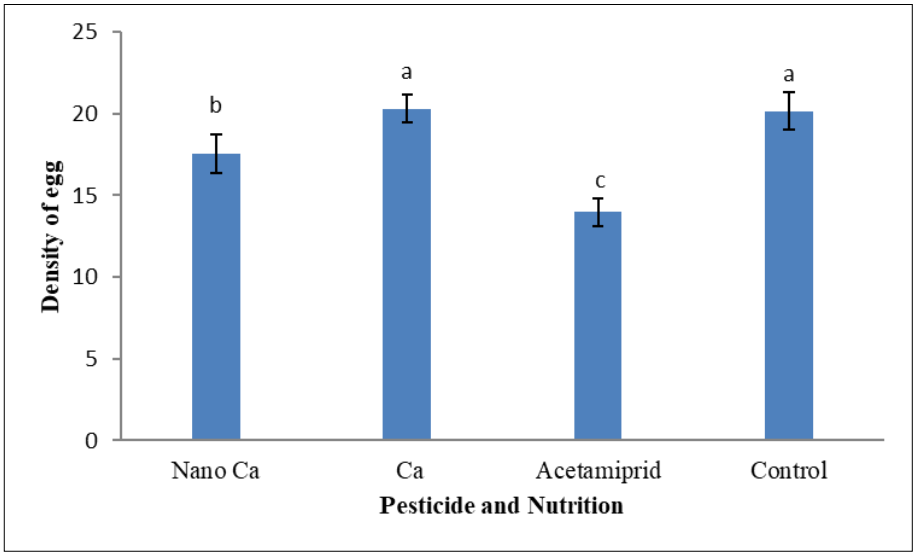


Fig 2: Efficacy of nano-calcium on the population of eggs of *A. pistaciae.*

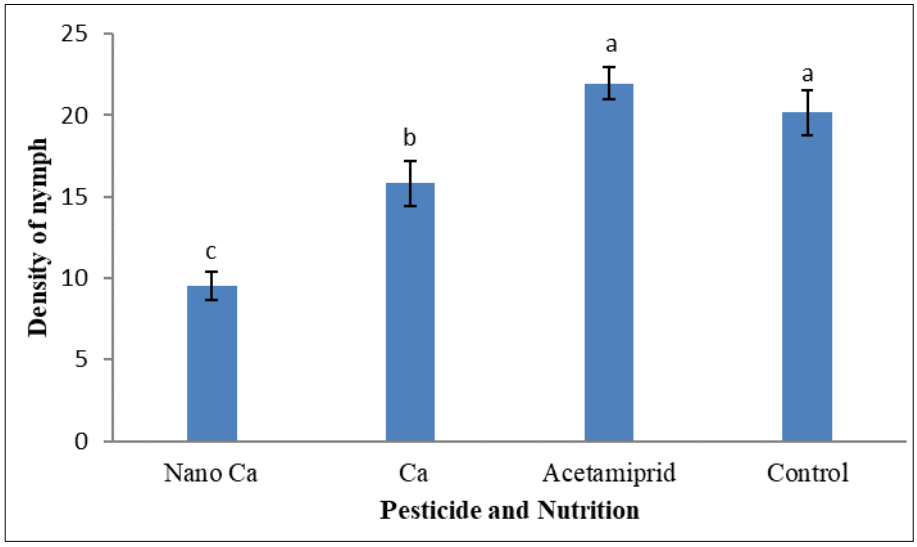


Fig 3: Efficacy of nano-calcium on the population of total nymphal of *A. pistaciae.*

Discussion

In this study, the effect of fertilization of nano-calcium on the density of *A. pistaciae* in comparison with the pesticide (acetamiprid) in pistachio orchards in Rafsanjan, Iran. There were no records of the influence of nano fertilization on pest densities, according to a global literature assessment. This research indicated that calcium might be used to help with *A. pistaciae* population management based on IPM program. Many scientists have looked at the impact of nutritional components on pests in their research [12, 15, 16, 17, 18] but there was no report on the effect of nutritional components in nanoscale.

These results showed that the application of spraying of acetamiprid had the greatest impact on egg abundance, whereas fertilization (Ca) had the greatest effect on the nymph population. As a result, it may be concluded that insecticides' effects are transient and that organic fertilizer can be used to substitute standard pesticides.

It can, therefore, be deduced that the effect of pesticides is impermanent and organic fertilization can be applied as a replacement for traditional pesticides. Whether the fertilizers can be used as an effective method of CPP's control and bring the populations under control following economic injury level or not. Our results indicated that Ca reduced the abundance of *A. pistaciae* but acetamiprid enhanced the population, which is consistent with earlier studies [12, 13]. The results of Luna [12] showed that the application of Ca on *Myzus persicae* and *Heliothrips haemorrhoidalis*, can decrease the density of sap-sucking pests such as aphids and mites. This might be due to calcium's location in the cell wall structure. The intensity of cell wall structure is affected by calcium content [19] and the content of calcium in leave cell wall structure impacts tissue susceptibility to fungal pathogenicity and pest attraction. [19, 20].

Our findings showed that fertilization of nano ca reduced the density of *A. pistaciae* more than fertilization of Ca in macroscale size. This may reflect the size of calcium, when the size of materials was reduced to the nanoscale, significant changes in their characteristics occurred [14].

Conclusion

In summary, the present study demonstrated that calcium decreased and acetamiprid increased the abundance of *A. pistaciae*. Our study suggests the use of calcium nanoparticles might reduce the density of *A. pistaciae* and could be useful as valuable tools in an Integrated Pest Management program for this pest.

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