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Research Article

Magnetite nanoparticles as a promising non contaminant method to control populations of fruit flies (DIPTERA: *Tephritidae*)

Abstract

"True fruit flies" belong to the family Tephritidae. Among them, the Mediterranean fruit fly (Medfly) *Ceratitis capitata* (Wiedemann) is the most economically important agricultural pest insect in the world. *Anastrepha fraterculus* (Wiedemann) is the South American fruit fly and represents a serious problem for countries of America. Both species share hosts fruits. Traditionally the control of fruit flies bases on the use of pesticides with chemical components. Due to their massive use to crops, pesticides are associated to environmental pollution and toxicity in mammals. An emerging technology is the use of nanomaterials with pesticidal activity or for the delivery of pesticides. The present paper reports: a) the synthesis of iron oxide (magnetite) nanoparticles and b) the effects of Fe_3O_4 nanoparticles during the development of the tephritid flies *C. capitata* and *A. fraterculus*. We sampled guava fruits to recover immature stages of fruit flies. Magnetite nanoparticles Fe_3O_4 were synthesized by co-precipitation of Fe (III) and Fe (II). We suspended doses of 100, 200 and 400 µg/ml of magnetite nanoparticles in water and we added the suspensions to larval medium. NPs are spherical with a medium diameter of 11 ± 2 nm and unimodal size distribution. During larval-pupal development, we checked out difficulties in the capacity to complete the natural biological cycle. Only 40% of larvae feeded in medium 400 µg / ml Fe_3O_4 NPs were able to continue their life cycle, in contrast to 92% of the control. Application of iron oxide (or magnetite) nanoparticles to larval food resulted in larvae toxicity expressed as dose-dependent lethality.

Keywords: larval-pupal development, pest insect, metamorphosis, *Ceratitis capitata*, *Anastrepha fraterculus*

Volume 8 Issue 4 - 2021

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Received: May 13, 2021 | **Published:** July 12, 2021

Introduction

"True fruit flies" belong to the Tephritidae family within DIPTERA. Only females damage fruits with their ovipositor while punching them out to lay eggs. The eggs hatch and larvae develop and feed inside fruits. Damage to fruits due to the punctures produced by oviposition translates into significant economic losses. The Mediterranean fruit fly, also known as medfly, *Ceratitis capitata* (Wiedemann) is the most important agricultural pest insect in the world on account of its economic impact, being regarded as one of the most invasive and highly polyphagous species infesting a wide range of fruit species. The South American fruit fly *Anastrepha fraterculus* (Wied.) has spread along the South and Central Americas' populations and have successfully established throughout several tropical and mild regions, due in part to an increased global trading activity which facilitates the diffusion of species. *A. fraterculus* has a broad host range, particularly in the family Myrtaceae within which, guavas serve as a reservoir of both species.[1–5] Traditionally, in pest control based on the use of pesticides with chemical components, the product applied directly to crops often reaches the urban environment. A considerable number of these pesticides are neurotoxic[6] and highly mutagenic.[7]

Historically, the control of *C. capitata* and *A. fraterculus* in Argentina was attempted almost exclusively by using a mixture of insecticides (generally malathion) and food attractants (such as hydrolyzed corn protein, sugar cane molasses, or hydrolyzed soy protein), commonly known as bait sprays. However, these mixtures continue to present a high level of toxicity to the ecosystem. Currently there is a growing interest in Argentina to combat both the medfly and the South American fruit fly through campaigns in which conventional chemical methods are gradually being replaced by ecological practices, such as the sterile insect technique (SIT). However, this requires the breeding of this species.

The indiscriminate, excessive and continuous use of chemical pesticides resulted in pests that acquired resistance to them. Due to these limitations of chemical pesticides and their adverse effects on humans, animals and soil (such as loss of fertility and the death of beneficial soil microflora), in recent years new agents have been rationally developed against pests from innovative, high-efficiency approaches. In this sense, the use of nanoparticles designed to face problems related to the agricultural sector has been investigated.[8] Recent studies revealed that these nano-agroparticles can act effectively not only as pesticides fungicides, insecticides, herbicides, pesticides) but also as plant growth-promoting factors. Nano-pesticides have shown a positive impact on the control of plant pests and diseases by delivering the active ingredient to the plant in a controlled way. This smart delivery provides sustainable solutions and reduces the amount and cost of fertilizers and pesticides for farmers. In addition, its reduction will be beneficial by allowing the health of the soil to improve, along with the nutritional quality of crop production.[9] Despite the fact that several types of nano-pesticides have been synthesized and their pesticidal activity has been proven, the incorporation of nanoparticulate systems for pest control must be carefully studied due to the harmful effects they could cause in humans and in the ecosystem. To overcome these possible toxic effects, research has been directed to the design of NPs with greater biocompatibility. The bio-applications based on the magnetite nanoparticles (Fe_3O_4 NPs) used here have received special attention because they offer unique advantages over other materials since they are cheap and easy to produce, physically and chemically stable, biocompatible, environmentally safe,[10] and they may be tuned and functionalized for specific applications. In this context, magnetite nanoparticles offer an option that combines both aspects. In this way, Fe_3O_4 NPs become very promising candidates for the study of their potential application in pest control.

For example, copper monoxide nanoparticles inhibit development and modify the absorption of nutrients in cotton plantations. On the other hand, the nanoparticles of zinc oxide, copper or cerium oxide produce translocation in shoots of carrot plantations. The use of silver nanoparticles[11] for the same purpose also compromises the health of living organisms, producing physiological anomalies, such as oxidative stress in the *Malva crispa* (Linn) species. It is very efficient in controlling the pest, specifically interfering with its larval development, and it does not generate toxicity at the environmental level; in fact, at high concentrations (1000 ppm) it is even efficient as a crop fertilizer demonstrated by the latest data we have registered in a work in which we use these same magnetite nanoparticles for the elimination of invasive species, such as chicory in corn plantations). It does not generate toxicity at ground level, which is ultimately the most important aspect since that is where the nanoparticulate system ends up; which, being an iron oxide, eventually serves as a fertilizer. The nanoparticulate size of the proposed system gives it superparamagnetic properties that offer the possibility of being extracted with a magnet, in the event that it reaches water courses. The synthesis of magnetite nanoparticles is relatively inexpensive; a final system in aqueous solution is obtained, with high yield and good performance in terms of its effectiveness.

Due to their massive use, the application of pesticides has been related to toxicity in mammals, environmental pollution, bio accumulation and were classified as human carcinogens according to the International Agency for Research on Cancer.[12] An additional challenge is the increasing frequency with which many insect species develop resistance to many of the active ingredients used as insecticides.

An emerging technology is the use of nanomaterials with pesticidal activity or for the delivery of pesticides.

Current research on new nanopesticides can be divided into two novel strategies, both of great interest today, such as the formulation of nanoemulsions with existing pesticides and the development of nanomaterials with pesticidal activity.[13,14] The development of nanopesticides in turn includes overcoming the main limitations of current strategies, developing formulations of remarkable stability, activity and penetration into the target organism, resistant to the defense of the plague, benign for plants and mammals and that is profitable.

Despite the fact that many of these nanomaterials appear to have high pesticidal,[13,15,16] as they are synthetic materials of usually inorganic composition, they cannot easily biodegrade and can accumulate or even be transported. NPs of silver, gold, palladium, platinum, titanium and zirconium have been synthesized from viruses, bacteria, actinomycetes, yeasts, fungi and plant extracts[17] for the control of various pests such as head lice, mosquitoes and flies.[18–20] With these concerns in mind, it has been chosen to find nanomaterials obtained from green synthesis, that is, from natural sources or by organisms, or nanomaterials with high bio-compatibility.[13] They possess biomedical applications,[21,22] environmental applications, they are biodegradable and can even act as a fertilizer (source of Fe) for plants.[23,24]

In this context, the unique properties of NPs have enabled to be used in diverse fields of biology.[25–27] Is crucial of research help discern the complex interactions between magnetite nanoparticles and animal model in relation to developmental changes, tissue-organ properties apart from behavioural differences.[15]

The main objectives of this study were to synthesize a simple, inexpensive and biocompatible nanomaterial such as superparamagnetic magnetite nanoparticles (Fe_3O_4 NPs) and to investigate the effects of these nanoparticles on successive immature stages of the most important fruit flies species along with their biological consequences.

Materials and methods

Sampling of fruits

On March 2019, 270 guava fruits (150 of *Psidium guajava* L. and 120 of *Feijoa sellowiana* L.) were collected in the Department of Fruit Production, Agronomy School and in the Veterinary School, Buenos Aires University, Argentina. All the fruits were placed in pots with sterilized sand. Larvae were progressively recovered and transferred to new pots with rearing food in order to perform present experiments.

Larval development

Larvae were fed on rearing medium. To eliminate possible effects of rearing medium on larval development, we tried two types of recipes: one using pumpkin and the other one using wheat germ. 100 mg larval food was poured into each pot. Each treatment consisted of two repetitions and each repetition equals 1 pot with 50 larvae.

Synthesis and characterization of Fe_3O_4 nanoparticles

Magnetite nanoparticles Fe3O4 were synthesized by co-precipitating Fe (III) and Fe (II). Analytical grade Iron (III) chloride FeCl3.6H2O (6.16g) and Iron (II) $FeCl_2.4H_2O$(3g) was dissolved in 100 ml of Milli-Q water and stirred vigorously at 90°C. Next, 10 mL of ammonia 25% were gradually dropped until the pH of the solution reached 10.

After an additional 30 min of stirring, it was allowed to cool to room temperature. The obtained magnetic particles are washed repeatedly with Milli-Q water using a magnet. Finally, the magnetic solid was dried for 24 hours at 70°C.

The morphology of the Fe_3O_4 nanoparticles was observed under a Zeiss SUPRA 40 scanning electron microscope (SEM). A LakeShore-7400 vibrating sample magnetometer (VSM) was used to perform the magnetic measurement of the products. Magnetic hysteresis loops were registered at room temperature in a field of 10,000 Oe to determine the samples' saturation magnetization (Ms). In order to determine the structural properties of the samples, an FTIR Nicolet 8700 spectrophotometer, with a resolution of 4 cm^{-1}, 64 scans using an ATR accessory, was employed. The structure and phase purity of the synthesized Fe_3O_4 NPs were investigated on a Siemens diffractometer D5000 using CuKα radiation (wavelength, λ¼1.5406 Å) with variable slits at 45 kV/30 mA to obtain patterns of X-ray powder diffraction (XRD).

Magnetite suspensions and treatments

Magnetite was suspended in water and mixed in a shaker. Suspensions of magnetite Fe3O4 nanoparticles were in the following concentrations: a) 100 μg/ml; b) 200 μg/ ml; c) 400 μg/ml. The treatments were: control (without Fe3O4 nanoparticles in the larval

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food) and 1 ml of each suspension -with the concentrations described- was added to each pot on April 4th.

Developmental stages recognition

The successive larval stages and metamorphosis stages prepupa, pupa and pharate adult, were examined under a Zeiss Stemi binocular magnifier.

Larval and pupae records

The first record of larvae was counted on the sixth day after magnetite treatment. Photos were taken under the magnifying glass using a Cannon G10 digital camera. Data were analyzed in the software SAS[28] version 9.2 where an ANOVA was performed and -for all the analysis-the criterion of statistical significance was defined $p<0.05$.

Results and discussion

Nanoparticles characterization

Synthesized iron nanoparticles are spherical (Figure 1A) with a medium diameter of 11±2 nm with a unimodal size distribution. The hysteresis loop of the Fe3O4 NPs is shown in Figure1B. The saturation magnetization (Ms) was 68.1 emu/g, a close value to that of bulk magnetite, indicating high superparamagnetic behaviour. The FTIR spectrum of the NPs (Figure 1C) exhibited the vibrational splitting-up peak at 551 and 582 cm^{-1} related to Fe-O bonds and attributed to magnetite octahedral and tetrahedral sites, respectively.[29]

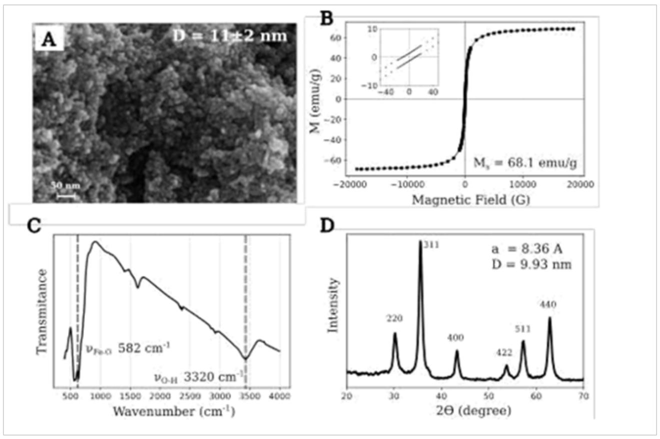

Figure 1 (A) SEM images, **(B)** VSM hysteresis loops measured at room temperature, FTIR spectra and **(D)** XRD patterns of magnetite NPs.

Hydroxyl groups located on the NP surface were detected in the wide absorption of O-H stretching at 3320 cm^{-1} for Fe_3O_4, and the peak at 1626 cm^{-1} is due to the result of the angular vibration of O-H. These signals are presented because the synthesis of NPs was carried out in water, so the surface of the material is covered by hydroxyl groups. The crystallinity of the magnetite sample was investigated by XRD as shown in Figure 1D. The patterns of the sample exhibited the six characteristic peaks of the inverse cubic spinel structure of Fe_3O_4 at 2θ of 30.18°, 35.56°, 43.28°, 53.74°, 57.32° and 62.86° that represent the corresponding indices of (220), (311), (400), (422), (511) and (440), respectively.

The calculated cell parameter (a) was 8.36 Å, a reasonable value for magnetite NPs due to its closeness to that of bulk magnetite (a = 8.396 Å). The calculation of crystal size using the Scherrer equation revealed that the average size of the magnetite NPs was approximately 10 nm, in agreement with the size determined by SEM.

Analysis of developmental stages under the effects of magnetite NPs

Magnetite NPs have affected immature stages of *C. capitata* and *A. fraterculus* life cycle. The brittle cuticle of many insects accounts for the alteration of the chitinization process during pupariation. As a consequence it was impossible to recognize to which one species belong the larvae and pupae recovered after magnetite treatments (Figures 2&3). Under controlled laboratory conditions, the life cycles duration of our reference strains are 30 days for *C. capitata Arg 17* and 45 days for *A. fraterculus fArg1* (Figure 2). If females from both fruit flies species oviposite at time cero, many developmental stages are overlapped (Figure 2). For example, larval stages, pre pupa and half the pupa stages of *C. capitata* are overlapped with the larval stages of *A. fraterculus*. Similarly, the other half of pupa stage and pharate adult stages of *C. capitata* overlap *Anastrepha*´s pre-pupa and pupa stages (Figure 2).

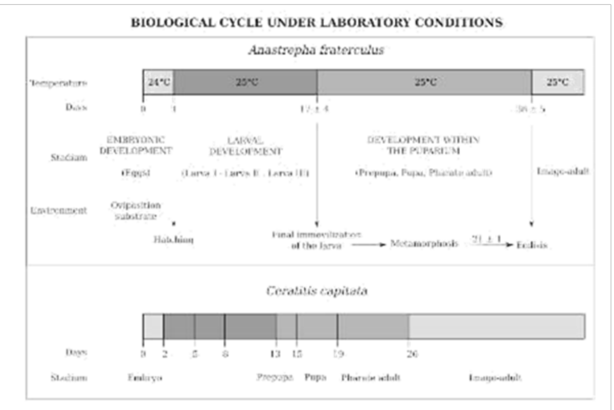

Figure 2 Biological cycle of *Anastrepha fraterculus* (Basso, 2003) and *Ceratitis capitata* under laboratory conditions.[35]

The brittle cuticle of many insects accounts for the alteration of the chitinization process during pupariation what makes impossible to properly identify the species (Figures 3 & 4).

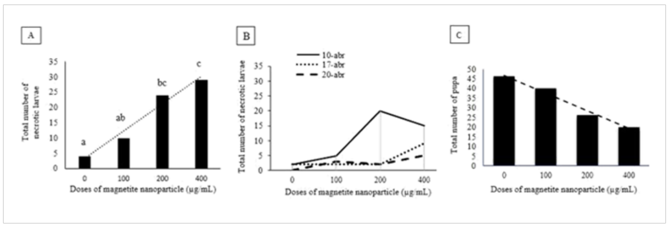

Figure 3 (A) Number of necrotic larvae under three different NPs suspensions (μg/mL): "0" is "control" *; **(B)** Number of necrotic larvae over doses of NPs measured in three different dates; **(C)** Total number of pupae under three different NPs suspensions. *Mean values followed by the same superscript letter are not significantly different (P > 0.05) by ANOVA Tukey's test.

First generation data from exposed immature stages

Analysis of exposed larvae and pupae to three different aqueous suspensions, showed that the higher the concentration of NPs, the greater the damage. Damage was measured from larval abnormalities verified in delayed growth and development (Figure 3A) as well as high (p=0.004) necrosis (Figure 3A–3C). During the larval-pupal development we checked out difficulties in the capacity to properly complete the natural biological cycle (Figures 3&4). We documented

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the presence of magnetite in the larval gut (Figure 4B). Only 40% of larvae feeded in medium 400 μg/ml Fe_3O_4 NPs were able to continue their life cycle. This is in contrast to 92% of the control treatment larvae that evolved to the pupal stage (Table 1). This result is first observed in Figure 2A where the mean value of dead larvae in the control treatment, is 8% (4 dead larvae/50).

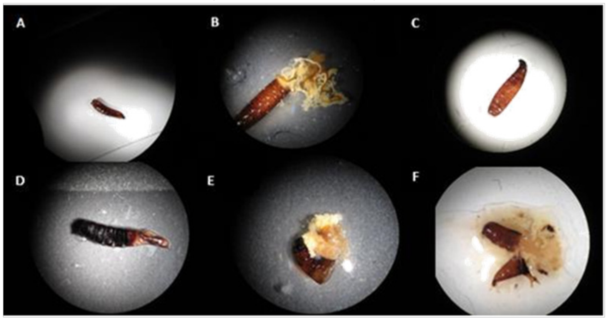

Figure 4 Effects of magnetite suspensions on larvae six days after NPs addition to the food. A: Treatment 100 μg/mL; B, C and D: 200 μg/mL; E and F: 400 μg/mL.

Another relationship that we can observe is in the treatment 400 μg/ml of magnetite Fe_3O_4, where 40% of pupae, less than half of the total (Table 1), showing statistical difference in relation to the other. Since the larvae did not move, they were dead or intoxicated; a general disorder of the metabolism of the epidermis that enters cell death takes place, one of the consequences being the tanification and/or melanisation (Figure 3). The level of oxidation in the epidermis of a dying bug causes phenol oxidases to oxidize everything (before this, the previous steps were regulated). There were any larvae moving because the cuticle was stiff. Those that reach the pupa stage are more behind in the poisoning: it is not a true sclerotization but an altered one. The main precursor of the sclerotization and pigmentation of insect brown cuticles is the *ebony* protein[30] or NBAD (N-Beta Alanil Dopamine-) synthase which catalyzes the synthesis of NBAD. Apart from its epidermal expression, immunodetection experiments show the novel localization of NBAD-synthase in different regions of the adult brain and in the pharate adult foregut.[30] Chen et al.,[31] found that after dietary uptake of chemical co-precipitation of Fe chlorides in an alkaline solution, *Drosophila* female flies are more likely to exhibit an adverse response, such as developmental delay in the egg–pupae and pupae–adult transitions.We found larvae difficulties in advancing to the next stage of development (Figures 3&4).

Table 1 Analysis of survival during larval-pupal transition after treatments under three different aqueous suspensions (μg/mL). Average number (A) and percentage (B) of pupae recovered from all repetitions after each treatment

NPs Dosis	**Control**	**A**	**B**
Treatment (μg/mL)	**Maximum value (total larvae)**	**Number of pupa**	**Percentage (%)**
Control	50	46	92
100	50	40	80
200	50	26	52
400	50	20	40

Ceratitis capitata and *Anastrepha fraterculus* are holometabolous insects with complete metamorphosis. Larval molt and metamorphosis within the puparium, are regulated by neurohormones ecdysone and juvenile hormone (JH).[32] In presence of enough levels of JH, ecdysone induces larval molts while sclerotization is arrested and does not start until this inhibition is canceled. When JH levels drops and molts are completed, pupariation takes place. Magnetite is producing larvae intoxication with the alteration in this regulatory machinery, ie pre mature and altered sclerotization of larval tissues without morphogenesis of the puparium (Figures 3&4). Magnetite altered larvae and pupae of both species.

Previous research works have reported acute intoxication of *Drosophila melanogaster* with iron ($FeSO_4$) and proved to diminish fly survival and locomotor activity, including climbing capabilities.[33] Chen et al.,[31] conclude that *Drosophila* uptake of NPs caused a significant decrease in the developmental delay at the egg-pupae and pupae-adult transitions. Additionally, Gorth et al.,[34] found that silver NPs significantly decreased the likelihood of eggs to pupate and reduced the percentage of pupae hatched to adults. Iron NPs added within fly food resulted in larvae toxicity expressed as dose-influenced lethality (Figures 4&5).

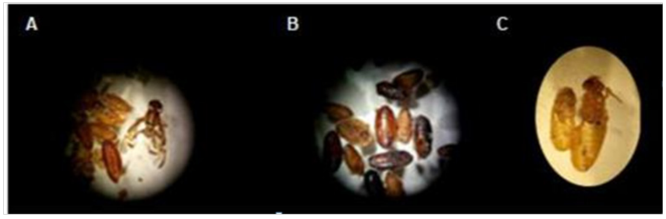

Figure 5 Behavioural and morphological evidence of Fe3O4 nanoparticles effects on pupae, pharate adult and adult fly. A) A. fraterculus adult fly; B) Pupae with altered puparium, C) Uncompleted ecdisis of pharate adult.

The toxicity of several NPs was accessed using *Drosophila melanogaster*. Pappus & Mishra[35] found that after oral route of exposure, the NPs enter the gut, cross the peritrophic membrane barrier and induce apoptosis.

We found that**,** in the gut, NPs (Figure 4B) produced toxicity which resulted in developmental delay, with a decrease in pupa count and fly hatching, along with weight loss. The adult fly hatched following magnetite NPs treatment showed phenotypical defects in different body parts and could not complete ecdisis (Figure 5C). In *Drosophila melanogaster* new born flies presented toxicity symptoms such as imperceptible movement and abnormal wing and bristle phenotypes after treatment with zinc oxide.[36,37]

Barik & Mirsha[38] studying the effects of silver nanoparticles (AgNPs) in *Drosophila* concluded that the NPs distresses larva to pupa and pupa to adult transitions. Overlapping of both species developmental stages along with magnetite damage made difficult to recognize if a larvae or a pupa corresponds to *C. capitata* or to *A. fraterculus* (Figures 3&4). Magnetite nanoparticles were produced and were tested to control populations of Tephritid fruit flies and results showed that they: a) disrupt the life cycle of both *C. capitata* and *A. fraterculus* by distressing the development and behaviour of these fruit flies; b) intoxication produced by magnetite treatment altered the phenotype of larvae and pupae (Figures 3 & 4) and disabled the ecdysis of pharate adult (Figure 5); c) magnetite nanoparticles were synthesized in a reproducible, simple and economic way and were characterized by several techniques. These results showed: 1- short-term exposure of a concentration of 100 μg/ml of Fe_3O_4 nanoparticles under laboratory conditions, is sufficient to cause harmful and lasting effects on the life cycle traits of both tephritid fruit fly species (*C. capitata* and *A. fraterculus*), which could also occur in other species and organisms. 2- The delay in growth and development, high necrosis and mortality of the larvae, along with phenotypic alterations such as abnormalities of wings and bristles, and the alteration and interruption of the life cycle, are in tune with the changes observed in model species such as *Drosophila melanogaster*, when exposed to nanoparticles.[36–40]

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Conclusions

Historically, the control of *C. capitata* and *A. fraterculus* in Argentina was attempted almost exclusively by using a mixture of insecticides (generally Malathion) and food attractants (such as hydrolyzed corn protein, sugar cane molasses, or hydrolyzed soy protein), commonly known as bait sprays. However, these mixtures continue to present a high level of toxicity to the ecosystem. Currently there is a growing interest in Argentina to combat both the medfly and the South American fruit fly through campaigns in which conventional chemical methods are gradually being replaced by ecological practices, such as the sterile insect technique (SIT), without. However, this requires the breeding of this species. The incorporation of the use of nanoparticulate systems for the control of insect pests in agriculture, must be carefully studied by virtue of the harmful effects that they could cause in humans and in the ecosystem. For example, copper monoxide nanoparticles inhibit development and modify the absorption of nutrients in cotton plantations. On the other hand, the nanoparticles of zinc oxide, copper or cerium oxide produce translocation in shoots of carrot plantations; The use of silver nanoparticles Armstrong et al for the same purpose also compromises the health of living organisms, producing physiological anomalies, showing for example oxidative stress in the Malva crispa Linn species.

In this context, magnetite nanoparticles offer an option that combines both aspects. It is very efficient in controlling the pest, specifically interfering with its larval development, and it does not generate toxicity at the environmental level; in fact, at high concentrations (1000 ppm) it is even efficient as a fertilizer for crop (the latter data that we are recording in a work in which we use these same magnetite nanoparticles for the elimination of invasive species, such as chicory in corn plantations). It does not generate toxicity at ground level, which is ultimately the most important aspect since that is where the nanoparticulate system ends up; which, being an iron oxide, it eventually serves as a fertilizer. The nanoparticulate size of the proposed system gives it superparamagnetic properties that offer the possibility of being extracted with a neodymium magnet, in the event that it reaches water courses. The synthesis of magnetite nanoparticles is relatively inexpensive; a final system in aqueous solution is obtained, with high yield and good performance in terms of its effectiveness.

Acknowledgements

None.

Conflicts of interest

No conflicts to declare.

Funding

None.

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