Nanostructured alumina as seed protectant against three stored-product insect pests

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Abstract

Nanoparticles represent a promising technology to enhance the efficacy of bioactive materials and a large number of studies showed the effectiveness of nanostructured materials against various arthropod species of economic importance. In this work nanostructured alumina (NSA) was prepared using sol-gel method and the effect of NSA was evaluated as seed protectant against the main seed-infesting insect pests *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae) *Stegobium paniceum* (L.), (Coleoptera: Anobiidae), and *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae). Besides, we tested the effects of NSA on seed germination and plant growth and finally, we assessed the presence of NSA as a contaminant in the leaves of bean plants germinated from NSA-treated seeds. The results showed significant insecticidal activity of NSA against the three tested species. After sixteen days, the percentage of insect mortality at the highest NSA concentration tested (400 mg Kg^{-1}) was 100.00% for *S. paniceum* followed by *T. confusum* (79.41%), and *O. surinamensis* (80.64%). Besides, *in-vitro* tests indicated that NSA has no effects on seeds germination and on radicle and shoot elongation. No effects of NSA were also observed in pot tests on the bean's plants. No differences were recorded in the leaves area, stoma density and roots length. On the contrary, the shoot of plants from NSA-treated beans was about 66% higher than the one of the non-treated plants (shoot, 15. 07 cm for the control and 22.76 cm for NSA-treated plants). Finally, no contamination by alumina particles was found by EDX-system coupled with Scanning Electron Microscopy (SEM) on the surface of the *P. vulgaris* leaves obtained from NSA-treated beans. Overall, the results showed that that NSA could be an effective protective agent for the control insect pests during the seeds storage.

Keywords: *Alumina nanoparticles; Sol-gel; Insecticidal activity; alumina characterization; stored seeds insect pest.*

1. Introduction

Insect pests constitute a major threat not only to stored food and grain (Jayas, 2012) but also to seeds for sowing. Further, due to their high economic value and low acceptable damage threshold, seeds for sowing require particular protection from insect pests resulting in the application of pesticides to overcome yield losses (Pimentel, 2019). These seeds, usually stored in relatively small quantities and in separate packages, are particularly susceptible to insect attack because of their prolonged period of storage, often more than one year (Stejskal, 2015). Moreover, due to the global market, seeds insect infestation promotes the spread of invasive stored food pests (Stejskal et al., 2014).

Currently, the protection of seeds against insect pests relies on mechanical (sieving to remove dust, impurities, and pests before accepting new seed batches into facilities) and on chemical treatments by phosphine, pyrethrins, pyrethroids, neonicotinoids and organophosphates (Stejskal et al., 2015). However, the massive use of synthetic pesticides led to the development of resistance in insect pests, as well as to severe consequences for human health and the environment (Guedes et al., 2006; Ribeiro et al., 2003; Desneux et al., 2007; Daglish et al., 2014; Naqqash et al., 2016).

Neonicotinoids, that are the most widely used class of insecticides worldwide (Longhurst et al., 2013) have been largely used for seed coating, showed to cause a variety of toxic effects to vertebrates including humans (Wang et al., 2018). Moreover, numerous evidences suggest that synthetic insecticides can seriously affect non-target species in natural and agricultural

ecosystems (van der Sluijs et al., 2015) such as pollinators (Tomè et al., 2012; Goulson, 2013), aquatic invertebrates and insectivorous birds (Van Dijk et al., 2013; Hallmann et al., 2014). For these reasons, the use of such chemicals is now under increasing restrictions worldwide (Handford et al., 2015) and new effective, environment-friendly tools for the control of seed-infesting insect pests that avoid the use of synthetic pesticides are strongly needed. In the last years the research has focalized on the use of natural substances such as aromatic plants essential oils and monoterpenes (Tapondjou et al., 2005; Benelli et al., 2015; Bedini et al., 2016; Bedini et al., 2017) and inert dusts (Mewis and Ulrichs, 2001; Lee et al., 2003; Athanassiou et al., 2005; Kljajić et al., 2010; Pierattini et al., 2019). In this regard, nanoparticles technology gave new possibility to manage seeds insect pests avoiding the use of synthetic pesticides (Kumar et al., 2010; Murugan et al., 2015; Athanassiou et al., 2018).

Nanostructured materials have been showed to have properties that are not shared by non-nanoscale particles with the same chemical composition (Auffan et al., 2009). The small size (1-100 nm), results in very large surface/volume ratio per unit weight (Paull and Lyons, 2008), increasing the toxicity of the bioactive substances. Actually, a large number of studies showed the effectiveness of nanoparticles against various arthropod species of economic importance (Athanassiou et al., 2018; Lazarević et al., 2018; Stadler et al., 2009; 2012; 2017; Buteler et al., 2015). Among nanostructured materials, nanostructured alumina (NSA), was showed to be effective as a contact insecticide (Debnath et al., 2011; Kitherian, 2017; Stadler et al., 2017). The present investigation was undertaken with aim to assess the potential of NSA synthesized by sol-gel method as crop-plant seeds protecting against seed-infesting insect pests.

For this purpose, we evaluated the effect of NSA-treated seeds on the main seed-infesting insect pests *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae) (Michael & Zimmerman 1990; David et al. 2013) *Stegobium paniceum* (L.), (Coleoptera: Anobiidae) (Doijode, 2012), and *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae) (Hagstrum &

Subremanyam, 2016). Besides, we tested the effects of NSA on seed germination and plant growth and, finally, we assessed the presence of NSA as a contaminant in the leaves of plants germinated from NSA-treated seeds.

2. Materials and methods

2.1. NSA preparation and characterization

NSA was prepared by the method described by Li et al. (2006) with little modifications. A preliminary solution was prepared by sol-gel method as follows: 0.5 M aluminium nitrate and 50 mL 1, 4-butandiol were gradually added to 200 mL 1:1 water-ethanol solution. Then, the solution was placed on a hot plate at 40 °C for 30 min. 0.55 mL Citric acid (0.55 mL) was dissolved in 40 mL deionized water, were added to the solution and continuously stirred until a colloidal solution was prepared. The obtained solution was heated in a water bath at 80 °C for 18 h to evaporate the solvent and then placed on a hot plate at 120 °C for 4 h until the viscosity and colour changed as the solution turned into a transparent stick gel. The obtained sol-gel precursors were then dried at 200 °C for 12 h in an oven and grinded into powders. Finally, the pale brown powder obtained after gel drying, was heated at 1000 °C for 1 h. The NSA obtained were characterized by X-ray diffraction (XRD) and Fourier Transform Infrared Spectroscopy (FT-IR spectroscopy) analyses. The XRD analysis is one of the most widely used, empirical evidence to estimate the crystalline size of nanoparticles (Guczi et al., 2003; Lemine, 2009; Pradeep et al., 2008; Sheng et al. 1998). The structure of NSA particles was investigated using a X'Pert Analytical type MPD multi-purpose X-ray diffraction system piloted by the X'Pert Hi Score software (Malvern Panalytical Ltd, Malvern, UK) to display, treat, index, and match the diffraction data to known phases (Degen et al., 2014).

121 FT-IR spectroscopy analysis of NSA was carried out using a IRAffinity-1 CE infrared
122 spectrophotometer (Shimadzu, Kyoto, Japan) over the range of 4000 to 400 cm^{-1}. The analysis
123 was carrying out at room temperature on KBr discs made up of 10 mg of NSA samples mixed
124 in about 150 mg of ground KBr (IR grade, > 99%). The powder was pressed into pellets (Ø =
125 10 mm) with low pressure (~ 1.5 psi) (Dablemont et al., 2008).
126
127 *2.2. Insect rearing*
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129 *Oryzaephilus surinamensis, S. paniceum,* and *T. confusum* were reared at room temperature,
130 65% relative humidity (RH), natural photoperiod, in PVC boxes (20 × 25 × 15 cm) containing
131 a mixture of chickpeas, beans, maize, and wheat grains and covered by a nylon net allowing air
132 exchange. Adults (about 7 days old) were used for the bioassays (Bougherra et al., 2015).
133
134 *2.3. Insecticidal activity of NSA*
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136 The insecticidal efficacy was tested mixing the NSA with the beans at the doses of 0 (control),
137 25, 50, 100, 200, and 400 mg Kg^{-1} of seeds. *Phaseolus vulgaris* L. var. "Piattelli" seeds were
138 shaken manually for 10 minutes with each dose of NSA in a glass jar (750 mL) to achieve an
139 even distribution in the entire seeds mass. Then, 10 unsexed adults of each insect species were
140 added into each jar. The jars were placed in an incubator at 27 ± 1°C, 60% RH and in the
141 darkness. The insects were considered dead when no leg or antenna movements were observed
142 after prodding them with a fine brush. Depending on the number of specimens available, five
143 (*O. surinamensis* and *T. confusum*) or three (*S. paniceum*) replicates for each NSA dose were
144 performed (78 experimental units, in total). The mortality percentage was then determined after

3, 6, 9, 12, and 16 days and adjusted by the Abbott's formula on the basis of the controls' mortality.

2.4. Effects of NSA on P. vulgaris

2.4.1. In vitro tests

Seeds of *P. vulgaris* var. "Piattelli" uniform in size were checked for their viability by suspending them in deionized water. The seeds were then surface sterilized in a 10% sodium hypochlorite solution for 10 minutes and rinsed through with deionized water several times. The NSA were dispersed in deionized water by ultrasonic vibration to obtain a concentration of 1.6 mg mL^{-1} and maintained in suspension by magnetic stirrer until use. A disc of Whatmann No. 1 filter paper was placed into a Petri dish (10 × 15 mm) and 10 mL of the NSA suspension were added. Ten seeds were then transferred onto the filter paper and the Petri dish covered and sealed with tape. The Petri dish was then placed in an incubator for 5 days at 25 ± 1°C in darkness. Control seeds were treated with 5 ml of deionized water only. The germination of the seeds was checked daily and the Germination Percentage (GP) was calculated according to Lombardi et al. (2019) by the following formula:

GP = (germinated seeds/total seeds tested) x 100.

P. vulgaris seedlings' root and hypocotyl elongation was measured at the end of the trial. Five replications for treatment were performed.

2.4.2. In pot tests

169 The effects of NSA on plant growth were investigated by pot culture. A 1.6 mg mL^{-1} NSA
170 solution was prepared as above reported. 150 mL (240 mg NSA) of the suspension were used
171 to wet a sterilized filter paper disk (10 cm∅). Seeds of *P. vulgaris* var. "Piattelli" were
172 immersed in a 10% sodium hypochlorite solution for 10 min to ensure surface sterility, rinsed
173 in deionised water and each seed was then wrapped in a sterilized filter paper charged with the
174 NSA solution. The wrapped seeds were then sown in pots containing 800 ml of substrate
175 (Universal Toprak Virgoplant, Leroymerlin, Livorno, Italy). Three seeds were sown in each
176 pot. As control, the seeds were wrapped in sterilized filter paper wetted by deionized water
177 only. Three replicates for each NSA treatment and the control (0 mg Kg^{-1}) were performed. The
178 beans seedlings were grown for 7 days in a grow chamber at 25°C (day) and 23°C (night), with
179 a 16:8 h light: dark photoperiod (photosynthetically active radiation intensity 280 μE s $^{-1}$ m^{-2})
180 and 60 ± 10% relative humidity. After 14 days root and shoot elongation were measured.
181
182 *2.4.3. SEM-EDX microanalysis*
183
184 SEM-EDX microanalysis were performed on NSA to confirm their composition and on the
185 leaves of the plants obtained from seeds treated with NSA to assess the contamination of the
186 plants by NSA. For the NSA microanalysis a little amount of the powder was adhered to an
187 aluminium stub using carbon tape glued on both sides and were directly put on the stage of the
188 electron microscopy chamber. Five lectures of the elemental composition were performed on
189 five different particles of the sample. As for the plant, five leaf samples (1 cm ^{2}) were cut from
190 fresh leaves of *P. vulgaris* var. "Piattelli" obtained from seeds treated or not treated with 240
191 mg NSA and grown as reported above. The samples were individually attached to aluminium
192 stubs using a carbon tape glued on both sides and were directly put on the stage of an
193 environmental scanning electron microscopy (ESEM) (FEI Quanta 200, Netherlands) and

194 analysed. The elemental analysis was obtained by an energy-dispersive X- ray analysis (EDX)
195 system performed in the ESEM chamber using a Bruker X-Flash 6/30 Detector. The chamber
196 pressure and accelerating voltage were 130 Pa and 10 kV, respectively.
197
198 *2.5. Data analysis*
199
200 The correlation between the mortality of the insect pest species and the NSA dose was
201 determined by univariate linear regression analysis. The insecticidal effect of NSA among the
202 species were investigated by a mixed model two-way analysis of variance with repeated
203 measures (RM-ANOVA). The RM-ANOVA model included the species as between-subjects
204 factor and the time of exposition as within-subjects variable with their interaction. The NSA
205 dose was considered as covariate and its effect was controlled in the analysis. Greenhouse-
206 Geisser correction was applied in case of violation of Mauchly's test of sphericity ($P < 0.05$).
207 The estimated marginal (EM) means of the insect pests' mortality are reported. Comparisons
208 between EM means were performed by pairwise comparison adjusted by Bonferroni correction
209 for multiple comparisons. Differences between plant germination, and plant growth data of
210 NSA-treated and NSA-non-treated samples were analysed by two-tailed student's t-test.
211 Percentage data (insect mortality and seeds germination) were arcsine transformed prior to
212 statistical analysis. All analyses were performed by SPSS 22.0 software (IBM SPSS Statistics,
213 Armonk, North Castle, New York, USA).
214
215 **3. Results**
216
217 *3.1. Alumina gel characterization*
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XRD alumina-gel analysis showed wide peaks corresponding to NSA (Figure 1). The average crystallite size of alumina powder obtained was 56.9 nm. Broad peaks at 37°, 43°, and 66° 2θ in the XRD spectrum indicated a conversion to slightly amorphous alumina (Table 1). All the detectable peaks can be attributed to the NSA.

The results of the FT-IR analysis are shown (Figure 2). The broad smooth absorption from 550 to 900 cm^{-1} reveals the formation of NSA. Significant spectroscopic strips at 1381, 1559, 1655, 1681 and 1736 cm^{-1} were identified as the absorption bands characteristic of H_2O and CO_2. Peaks localized at 3400 and 3500 cm^{-1} were assigned to stretching vibration and deformation vibration of liaison O-H.

3.2. Insecticidal effect of NSA

The toxicity assay of NSA showed a strong dose-effect relationship between NSA doses and insect mortality (*O. surinamensis*: $R = 0.684$; $F_{1,13} = 11.426$; $P = 0.005$*; S. paniceum*: $R = 0.952$; $F_{1,10} = 95.909$; $P \leq 0.001$; *T. confusum*: $R = 0.758$; $F_{1,13} = 17.535$; $P = 0.001$) (Figure 3A, 3B, 3C). After sixteen days, the percentage of insect mortality at the highest NSA concentration tested (400 mg Kg^{-1}) was 100.00% for *S. paniceum* followed by *T. confusum* (79.41%), and *O. surinamensis* (80.64%). Repeated measures ANOVA showed a significant within-subjects effects of the time of exposition on mortality of the three specie after controlling for the effects of the NSA dose ($F_{3, 152} = 94.233$; $P < 0.001$; $\eta_p^2 = 0.623$) with a significant interaction of the time x dose ($F_{3, 152} = 14.991$; $P < 0.001$; $\eta_p^2 = 0.208$) and of the time x species ($F_{3, 152} = 6.587$; $P < 0.001$; $\eta_p^2 = 0.188$). The effect of the species (between-subject effect) was significant also ($F_{2, 57} = 12.689$; $P < 0.001$; $\eta_p^2 = 0.308$). The estimated marginal means (evaluated at NSA = 151.23 mg kg^{-1}) showed that the most susceptible species was *S. paniceum* (mean = 36.08%) followed by *T. confusum,* and *O. surinamensis* (mean = 28.55, and 23.04, respectively) (Table

2). Pairwise comparisons of EM means showed that *S. paniceum* was significantly more susceptible to NSA then the other two insect species (Table 2). Median lethal concentration (LC_{50}) values calculated by Probit analysis were 61.53, 14.87, and 127.17 mg Kg^{-1} for *O. surinamensis, S. paniceum,* and *T. confusum*, respectively (Table 3). Consistently with the RM-ANOVA, RMP analysis showed that*, S. paniceum* was significantly more susceptible to NSA than *O. surinamensis* (*S. paniceum vs O. surinamensis* RMP = 0.242; 95% CI: 0.034; 0.770) and *T. confusum* (*S. paniceum vs T. confusum* RMP = 0.117; 95% CI: 0.010; 0.403).

3.3. Effects of NSA on P. vulgaris

The NSA treatment of the beans did not affect the *P. vulgaris* germination (t_8 = 1.159, *P*=0.192) nor the seedlings' root and hypocotyl elongation (t_8 = 1.074, *P* = 0.341; t_8 = 1.159, *P* = 0.280, respectively) (Table 4).

No negative effect on the beans plants was observed also in pot culture. After 14 days from the seedlings emergence no differences were recorded for the leaves area, stoma density, and root length (t_4 = 2.008; *P* = 0.115; t_4 = 2.456; *P* = 0.070; t_4 = 0.640; *P* = 0.557, respectively). On the contrary, we observed a positive effect of the NSA on the shoot growth (t_4 = 2.974; *P* = 0.041) with the treated plants that were about 66% higher than the non-treated plants (Table 5).

3.4. SEM-EDX microanalysis

The chemical analyses of the particles showed that the main element was aluminium (51.0 ± 0.1%) followed by oxygen (46.6% ± 1.2) and carbon (2.2 ± 0.3%).

The possible contamination of *P. vulgaris* plants by the NSA used for the seeds treatments was checked by SEM-EDX. The analysis carried out on the bean leaves revealed the presence

of elements constituents of the plant's tissues (oxygen, 66.6 ± 1.4; carbon, 33.2 ± 2.3; potassium, 0.2 ± 0.1%) while no evidence of NSA was found.

4. **Discussion**

The results of the X-ray diffraction (XRD) analysis consisting in well-resolved peaks, confirmed the polycrystalline and monophasic nature of the prepared material. The diffraction peaks provided a clear evidence of the formation of NSA with an average particle size of 56 nm. The elemental analysis of nanostructured alumina by energy-dispersive X-ray analysis (RDX) of nanostructured alumina confirms the presence of Al, O and C with mass percentage 34.83%, 33.97% and 31.19%, respectively. Rogojan et al. (2009) demonstrate that crystallite sizes obtain from X-ray diffraction images for the alumina powders using sol-gel methods is 10.70 nm. The differences between two results can probably explained by measuring the angles and intensity of the diffracted rays, the symmetries of the crystal structure (space group) and a three-dimensional image of the electron density in the lattice. From this density, the average position of the atoms of the crystal forming the crystal pattern can be determined as well as the nature of these atoms, their chemical bonds, their thermal agitation.

The result of FTIR allowed to clarify the structure–spectral relationships of the associated molecular vibrations, the resulting peak bands at 550 to 900 cm^{-1} can be attributed to the formation of alumina-oxygen. Janbey et al. (2001) reported that IR spectra shows the strong absorption bands at 1600 cm^{-1} due to the various vibration made of triethanolamine the metal ions also the bands appearing at 1005 and 800 cm^{-1} could be attributed to the presence of nitrates ions and bands appeared in the region 700-400 cm^{-1} could be the results of some trace amounts of metal oxides.

293 The toxicity bioassay showed the NSA is able to exert a significant insecticidal activity
294 against the main seeds pests *O. surinamensis*, *S. paniceum,* and *T. confusum.*
295 According to our results, the most susceptible species to the NSA was *S. paniceum*, while *O.*
296 *surinamensis* resulted the most resistant one.
297 Although no previous studies are available on the effects of NSA on the species tested in
298 this work*,* in line with our results Stadler et al. (2009) reported 100% mortality of the stored
299 grain pests *Rhyzoperta dominica* (Coleoptera: Bostrichidae) and *Sitophilus oryzae* (Coleoptera:
300 Curculionidae) adults in wheat treated with 1000 mg $kg^{-1}$of NSA dust after 9 days of exposure
301 and about 95% mortality after only 3 days of exposure. A high effectiveness of NSA was
302 observed also by Goswami et al. (2010) with about 90% of mortality of S. *oryzae* and *Tribolium*
303 *castaneum* (Coleoptera: Tenebrionidae) exposed for 7 days to 2000 mg $kg^{-1}$of hydrophilic NSA.
304 Similarly, NSA produced by combustion of glycine and aluminium nitrate caused more than
305 94% mortality of *S. oryzae* adults after 15 days of exposure applied on wheat at doses ranging
306 from 62.5 to 1000 mg kg^{-1} (Stadler et al., 2012). Nevertheless, the efficacy of this NSA for
307 control of *R. dominica* adults resulted in lower overall mortality levels than for *S. oryzae*.
308 Similar results were obtained when three novel NSA dusts, based on chemical solution
309 methods, were applied on wheat for control of *R. dominica* and *S. oryzae* (Buteler et al., 2015)
310 and Stadler et al. (2017) obtained a LC_{50} of 79.91 mg Kg^{-1} after 39 days of exposition with a
311 LT_{50} of 23.82 days when tested at 500 mg kg^{-1} for NSA against *S. oryzae*.
312 The insecticidal effect of the NSA could be probably due to the electrically charged
313 particles resulting by the oxidation of aluminium. Alumina electrically charged particles,
314 showing interaction between dipole-dipole promote the formation of aggregates that stick
315 firmly to the insect cuticle wax layer and generated electric charges resulting by triboelectric
316 effect (Pimentel, 2005). In addition, according to Stadler et al. (2012) and Buteler et al. (2015)
317 the toxic effect of the NSA, similarly to those of other insecticidal dust such as diatomaceous

318 earth should be due to the absorption by NSA of the epicuticular lipids, causing the insect death
319 by dehydration. The insecticidal effect should also depend on NSA physical characteristics,
320 (i.e. particle size, particle morphology) and on other biotic and abiotic factors such as target
321 species, and relative humidity (Stadler et al., 2012; Buteler et al., 2015).
322 We observed no significant effect of NSA on plant germination, seedlings elongation and
323 plant growth, nor we observed any aluminate contamination on the surface of the *P. vulgaris*
324 leaves.
325 Nanoparticles can penetrate into the plant through the stomatal openings or the bases of
326 trichomes and then transferred to various tissue (Fernández and Eichert, 2009). Previous
327 studies showed that nanoparticles whose diameter is less than the pore diameter can reach the
328 plasmatic membrane and cross it with incorporated transport carrier proteins or through ionic
329 channels interfering with metabolic processes (Jia et al., 2005) and reaching mitochondria or
330 nucleoli in both plant and insect tissues (Yasur and Rani 2013, 2015). For this reason, it is
331 important to assess the phytotoxicity of nanomaterials. Lee et al. (2010) evaluated the effect
332 of four metal oxide nanoparticles, aluminium oxide (nAl_2O_3), silicon dioxide ($nSiO_2$),
333 magnetite (nFe_3O_4), and zinc oxide (nZnO), on the development of *Arabidopsis thaliana* (L.)
334 Heynh (Brassicaceae) (seed germination, root elongation, and number of leaves) and, in
335 accordance with our results, found that aluminium oxide nanoparticles were no toxic whereas
336 they observed a toxic effect of the other three metal oxide nanoparticles. The absence of effect
337 of aluminium oxide nanoparticles on seed germination and root growth was also observed by
338 Lin and Xing (2007) who showed that the nanoparticles have no adverse effects on California
339 red beans, while Mahajan et al. (2011) observed a positive effect on seedlings growth of
340 *Vigna radiata* (L.) Wilczek, and *Cicer arietinum* L. (Fabaceae) after a treatment by ZnO
341 nanoparticles.

342 In this experiment, the only effects of the NSA we observe is the increase of shoot growth
343 in the treated plants that were about 66% higher than the non-treated ones (length of the shoot
344 15. 07 ± 1.20 cm for the control and 22.76 ± 2.25 cm for NSA treatment). In line with our
345 results, Khodakovskaya et al. (2009) observed that nanostructured carbon determines an
346 increase on tomato plants seed germination and the plant growth and, according to the authors,
347 such a positive effect of the nanoparticles could be due to their ability to penetrate the seed coat
348 and enhance the water uptake.
349 Nanotechnology has a huge potential to develop alternative pest control strategy. In this
350 study, we showed that alumina nanoparticles synthesized by sol-gel method could be an
351 effective protective agent that can be used to control the infestations of insect pests during the
352 seeds storage. Recently, the international community has paid great attention to issues of
353 environmental sustainability. In particular, the target 3.9 of the ONU 2030 Agenda for
354 Sustainable Development is aimed to substantially reduce, by 2030 the number of deaths and
355 illnesses from hazardous chemicals and air, water and soil pollution and contamination. Even
356 if further studies are needed to evaluate the efficacy of NSA seed treatments under a wide range
357 of applicative conditions our results showing the efficacy of NSA against the insect pest species
358 and the absence of negative effects on the seeds germination and plant growth indicate that
359 NSA may be a valid alternative to the chemical synthetic insecticides currently used for seeds
360 coating.

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362

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587 Tables

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Table 1. X-ray diffraction (XRD) analysis of the nanostructured alumina.

Peak position[a]	B structure[a]	Crystallite size[b]
25.58	0.167	48.7
35.15	0.143	58.4
37.80	0.123	35.1
43.34	0.143	59.9
52.54	0.143	62.0
57.48	0.163	55.5
61.29	0.122	36.4
66.50	0.143	66.5
68.19	0.122	78.4
77.22	0.061	68.1

589 [a], °2θ Th; [b], values are expressed in nm

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Table 2. Adjusted estimated marginal (EM) means of the mortality of *Tribolium confusum*, *Oryzaephilus surinamensis*, and *Stegobium paniceum* exposed to nanostructured alumina (NSA).

Species	Mean ± SE	95% Confidence Interval	
		Lower bound	Upper bound
T. confusum	28.556 ± 1.628 b	25.296	31.816
O. surinamensis	23.037 ± 1.628 b	19.777	26.297
S. paniceum	36.083 ± 2.016 a	32.046	40.121

Data are expressed as mean mortality percentage ± standard error. Covariate (NSA dose) was evaluated at NSA = 151.23 mg kg^{-1}. Different letters indicate significant difference by pairwise comparison adjusted by Bonferroni correction for multiple comparisons.

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Table 3. Median lethal concentration (LC_{50}) of nanostructured alumina (NSA) effective against adults of *Oryzaephilus surinamensis*, *Stegobium paniceum,* and *Tribolium confusum.*

Species	LC_{50}[a]	Intercept	*P*
O. surinamensis	0.44(0.23-1.02)	1.186	< 0.001
S. paniceum	1.10(0.60-10.70)	-0.138	0.544
T. confusum	127.17(60.24-305.92)	-0.978	0.001

[a], Concentration of the NSA that kills 50% of the exposed insects. Data are expressed as mg Kg^{-1}; in bracket, confidence interval. Pearson goodness of fit test: $\chi 2 = 3.379$; df = 11; $P = 0.985$.

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Table 4. Effects of nanostructured alumina (NSA) on seeds germination, and radicle and shoot elongation of *Phaseolus vulgaris* seedlings.

	Control	**NSA**
Seed Germination[a]	7.60 ± 0.60	6.00 ± 0.95
Ipocotyle[b]	30.12 ± 7.71	18.00 ± 7.06
Root[b]	5.20 ± 0.78 a	10.68 ± 5.04 b

[a], % of germinated seeds; b, cm; Control, seeds not treated with NSA. Data are expressed as means ± standard error.

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Table 5. Effects of nanostructured alumina (NSA) on plant of *Phaseolus vulgaris* grown in pot culture.

	Control	***NSA***
First internod[a]	2.59 ± 2.13	2.13 ± 0.39
Second internod[a]	5.76 ± 0.79	5.75 ± 0.66
Third internod[a]	6.70 ± 1.00	7.37 ± 0.50
Fourth internod[a]	0.67 ± 0.66 a	6.93 ± 1.25 b
Shoot (total)[a]	15.07±1.20 a	22.76 ± 2.25 b
Leaves area[b]	56.93 ± 3.15	64.13 ± 1.72
Stoma density[c]	15.58 ± 0.36	13.25 ± 0.88
Root[a]	12.61 ± 1.91	14.08 ± 1.28

[a], cm; [b], cm^2; [c], No. stoma cm^{-2}. Data are expressed as means ± standard error. Different letters indicate significant difference between treatments (t-test, $P < 0.05$).

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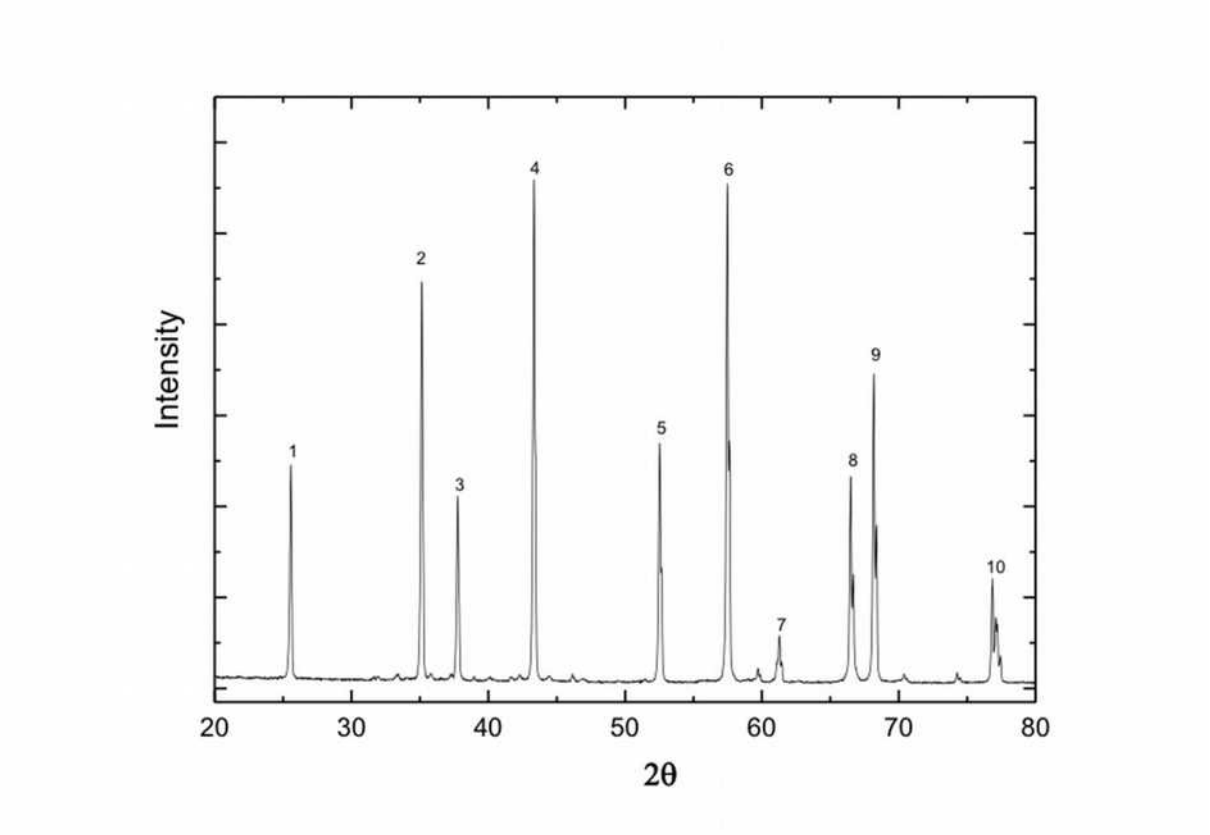

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656 **Figure 1**. X-ray diffraction (XRD) spectra of the nanostructured alumina (NSA) particles

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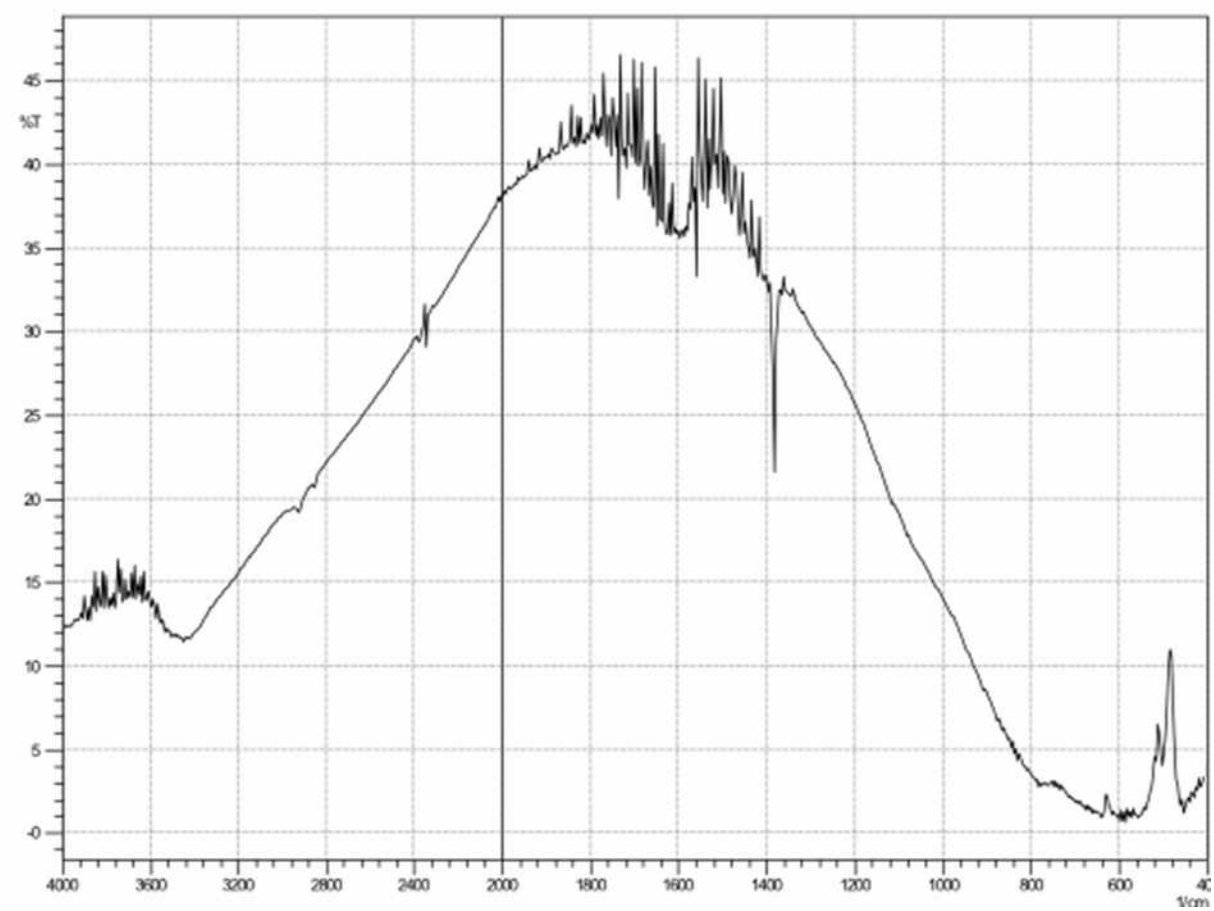
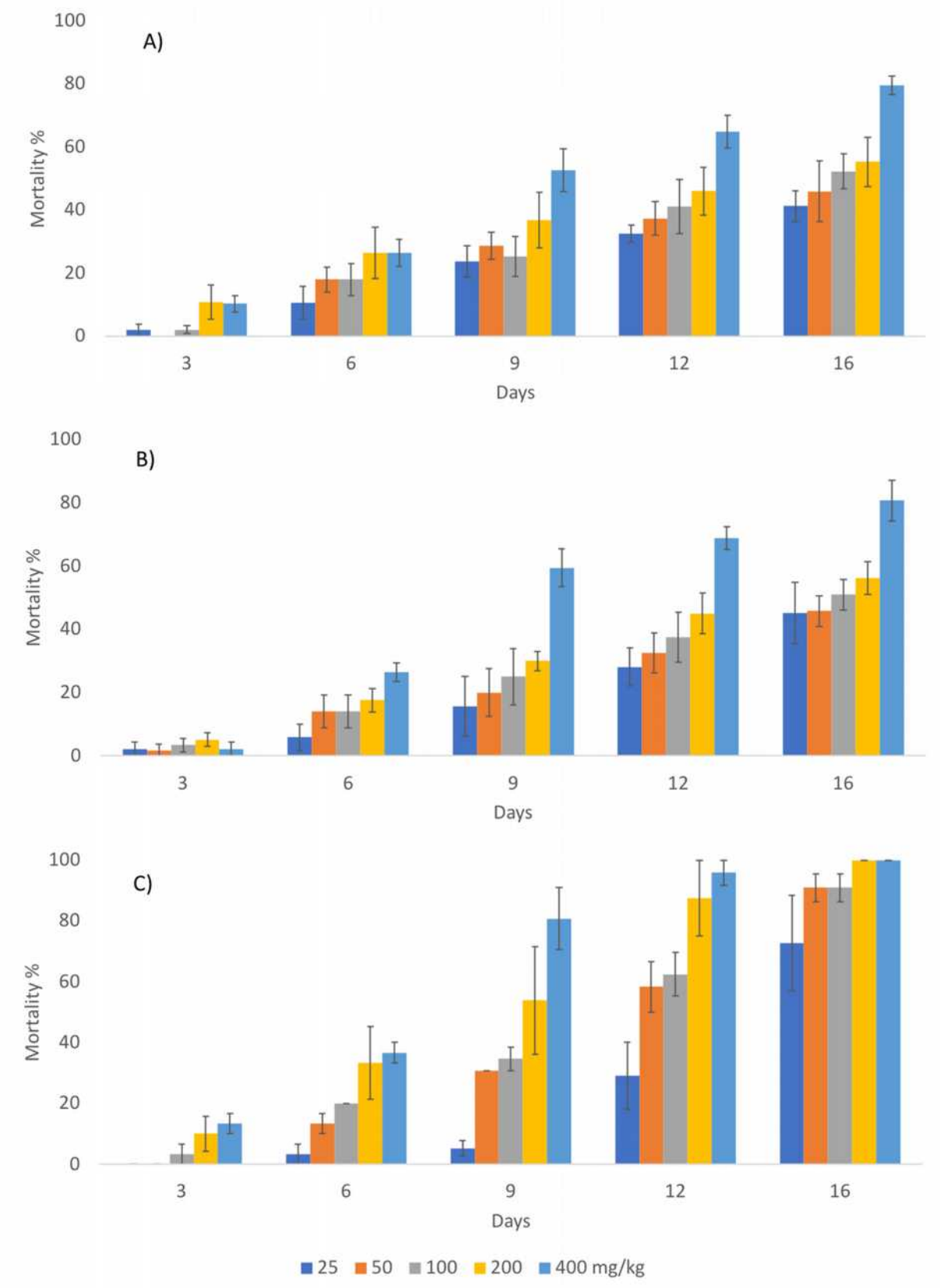


Figure 2. Fourier Transform Infrared Spectroscopy (FT-IR spectroscopy) spectra of nanostructured alumina (NSA) particles.



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670 **Figure 3.** Mortality (%) (mean ± SE) of *T. confusum* (a) and *O. surinamensis* (b) *S. paniceum*

671 (c) adults fed on beans treated with nanostructured alumina (NSA) particles.