

Impacts of green synthesized Silver-Nanoparticles against *Tribolium castaneum* (Coleoptera: Tenebrionidae)

Kanwal Shahzadi^{1*}, Farwa Bashir^{1*}, Kiaenat Nazir¹, Safora Seemab¹, Muhammad Afsar Ali Shaker², Irum Waheed¹, Asad Shehzaib³, Kainat Fatima¹

¹Department of Zoology Wildlife and Fisheries, University of Agriculture, Faisalabad.

²Department of Entomology, BZU Bahadur sub-campus, Layyah.

³Institute of Horticulture Sciences, University of Agriculture, Faisalabad.

*Corresponding Authors E-mails: Kanwalrao2526@gmail.com, Farwabashir17@gmail.com.

Abstract: Synthetic insecticides have been mainly used for the control of stored products insect pests. But have now becoming limited due to many drawbacks. Nano sciences and nanotechnology are the study of extremely small things at nm scale. The present study was carried at Chemistry Laboratory, Punjab Bioenergy Institute, University of Agriculture Faisalabad. *Tribolium castaneum* were collected from grain market, located in Faisalabad and population of insect was acclimatized to the laboratory. After extraction of plant materials (oils from *Ricinus communis* and *Citrus paradise*) biosynthesis of nano-particles was done accordingly to standard procedure. Toxicity bioassays was done by three concentrations (5, 10 and 15 %) of the plant oils (for each of the simple plant oil) and 100, 200 and 300 ppm (for nano-particles) were used. Data regarding mortality was recorded after 24, 48, 72 and 96 hours of the treatment application. In case of plant oil, highest mortality (36.12%) was recorded by *R. communis* at 15% and after exposure period of 96 (hr.) while *R. communis* silver nanoparticle gave 41.40% at 300 ppm. Repellency bioassay was done by area preference method. Silver nanoparticles repellency highest range was 67.89% at 15% concentration of *R. communis* and lowest was 28.31% at same concentration of the *C. paradise* oils, used. Hence, plant based insecticides can be helpful for the management of stored commodities insect pests.

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Introduction

Nano sciences or nanotechnology are the study of small things at nm scale, it is used across the all science fields, such as chemistry, Biology, physics, material sciences and engineering. Nano sciences is an emerging and vastly developed form that can encompasses the fundamental's elements which can understand and advanced arising form of exploitation of materials, which have one dimension. It has an ability to control individual atoms and molecules. It is the one of the most effective technology in recent decades (Hao *et al.*, 2011). Nano particles can be characterized into following parts such as Quatum dots, organic nanoparticles or metal oxides (Al_2O_3 , In_2O_3 , NiO, TiO_2 , ZnO, ZrO_2 , SnO_2 , CuO, MgO, Cu_2O , La_2O_3 , CeO_2), carbon nanotubes and fullerenes are also part of this and inorganic or metals (Al, Co, Ni, Fe, Au, Mo, Zn, Ag, Ti, Bi, W) (Rajput *et al.*, 2016). Store grains and their products are attacked different insects which can cause huge losses. (Ahmadani *et al.*, 2007). Storage of cereals is about to 9% losses occurred in developed countries and 20% in more developing countries (Phillips and Throme, 2010), it effects on the qualitative and quantitative loses of grains (weaver and Subramanyam, 2000). Postharvest loses range from 10 to 25% throughout the

world due to infestation of insect pests and microbial deterioration (Mathews, 1993). Wheat is the staple food of Pakistan, also cultivated all over the world expect the Antarctica (Taylor and Koo, 2012). Globally, Pakistan is the 3rd largest food crop and eight in world-wide wheat producing country (Shuaib *et al.*, 2007). Due to less efficiency of traditional method can't control the insect pests to damage the store grained crop therefore now we can use the advance technology such as nano materials. Nanomaterials hold great promise regarding their application in plant protection and nutrition due to their size-dependent qualities, high surface-to-volume ratio and unique optical properties. A wide variety of materials are used to make NPs, such as metal oxides, ceramics, silicates, magnetic materials, semiconductor quantum dots (QDs), lipids, polymers, dendrimers and emulsions. Now we can use the advance technology such as zinc and titanium oxide nanoparticle (Niemeyer and Doz, 2001; Oskam, 2006; Puoci *et al.*, 2008). Green synthesis of silver nanoparticles is the subdivision of nanotechnology. Recently, biosynthetic strategies employing either biological microorganisms or fungus or vegetation extract have emerged as an easy and feasible opportunity to greater complex chemical synthetic techniques to attain nanomaterials

(Logeswari, Silambarasan, & Abraham, 2015). Now a day's green synthesis of nanoparticles is one of the most interesting scientific areas of inquiry. The world health organization (WHO, 2017) has facilitated the use of biopesticides which are less expensive, effective and environmental friendly (Ullah, Ijaz, Mughal, & Zia, 2018).

Materials And Methods

Insects Rearing

Different and diverse age *T. castaneum* collected through grain market which is situated in Faisalabad. The population for all of the tow insect assimilate to the laboratory and 1.5 kg capacity having commodity in plastic jars (firstly decontaminate the store grain flour for *T. castaneum*, it can decontaminate for 30 minutes in 70°C through the oven (Lab Line Instrument Inc. Model No.3512-1) and it shielded through muslin cloths. Both insects' after three days from commodity adults will be sieved out. Target insects can have eggs which can be sieved commodities, will be placed in jars and it can place in an optimal condition (65±5 % R.H. and 30±2 °C) and it can be homogeneous and also getting the F₁ population (Hbib-ur-Rehman, 2018).

Collection and preparation of plant oils

Firstly, we can collect the leaves of *R. communis* and *Citrus paradise* from different localities in Faisalabad, the sample leaves was thoroughly washed with tap water followed by distilled water to remove the impurities. The wet plant leaves were kept on shade for 25 days and put in air dry. Dried leaves will be converted into powder form through the electrical grinder (Pascal engineering Co. Ltd., Gatwick road crawley Sussex, England) and it will be sieved through a mesh (40mm) then we acquire a fine powder form. After, plants leaves powder material extract were put in the Soxhlet apparatus by dipping or mixing 100ml of the methanol, chloroform, petroleum ether and n-hexane for 24 hours at 220 revolution per minute (Sagheer *et al.*, 2013).

Preparation of green synthesis Agnps

The method of (Murugan *et al.*, 2015) was used for the preparation of green synthesis of silver nanoparticles from the crude extract for the above mentioned four plant parts/material. Ten gram of dry crude extract powder was dissolved in 250 ml sterile distill water and the solution was boiled for 5 min. This solution/extract was filtered with Whatman filter paper No.1. The 1m M of silver nitrate (AgNO₃) solution was prepared by adding 1mM silver nitrate salt in 100 ml double distill water and mixed well until silver nitrate (AgNO₃) was dissolved. The 80 ml of silver nitrate (AgNO₃) solution was placed on hot plate magnetic stirrer and 20 ml of the proper made plant extract was added drop by drop. This solution

was boiled for 5 minutes. The color of a solution change was observed, that resulted in a brown solution, indicated the formation of silver nanoparticles. The solution was placed in falcon tubes and centrifuged at 5000 rpm for 15 minutes. The solution of green synthesized silver nanoparticles was converted into pallet forms, extra solution was removed from falcon tubes and the pellets were shifted into china dish (100 mm). The china dish was placed in oven at 50°C for 24 hours, drying the pellets of green synthesized silver nanoparticles. After drying, the pellets were grinded with pistol mortar. Powder was saved in Eppendorf tubes and tagged with proper name. The Eppendorf tubes were covered with aluminum foil for stability of green synthesized silver nanoparticles.

Efficacy of green synthesized silver nanoparticles against *Tribolium castanum*

The experiment was conducted with 3 treatments and 5 replications for synthesized silver nanoparticles from *R. communis* and *Citrus paradise*. To determine lethal concentration of green synthesized nanoparticles solution, serial dilution ranging from 100, 200 and 300 ppm were prepared. The required amount of adult were directly collected from field and put into the clean, air dried petri plates, Wheat grains were coated with different concentrations of silver nanoparticles solution and air dried. The control grains were treated with water alone. After every 24 hrs, 48hrs, 72hrs and 96hrs data of mortality was recorded.

Repellency bioassays

Repellency bioassays of plant extracts against test insect (*T. castaneum*) was carried out by area preference method. Different concentrations of plant oils (ethanol acetone based) viz., 5%, 5%, 10% and 15% of were sprayed on one half of the each filter paper. Whilst other half of each filter paper, were used as control unit (treated only with solvent alone (acetone)). Both the untreated and treated halves were stapled from center and were placed in Petri-dishes. 20 adult beetles were released at the joining point of both half of each filter paper. Three replica of individually treatment and control unit were used and number of bioassayed on the both areas (two half paper disks) were counted after 24 hours of the post treatment.

Data analysis

The collected data were analyzed by using static software 8.1. Treatments means were compared by using Tukey-HSD test at $\alpha = 5\%$.

Efficacy of green synthesized silver nanoparticles from *R. communis* and *Citrus paradise* against *Tribolium castanum*

To evaluate the mortality of *T. castaneum*, homogenous adults were released on treated diet in small plastic jars. Adults were allowed to feed on treated diet and data regarding mortality was recorded.

Wheat grains were used as diet and three concentrations of each plant extract were used viz., 5, 10 and 15%. Mortality data was recorded for 24, 48, 72 and 96 h of exposure period. For mortality

assessment insects were kept in incubators at 30 ± 2 °C and 60 ± 5 % RH. Each treatment and control were replicated three times.

Table 1: Percent mortality (Mean) of *T.castaneum* adults treated with plant oils under store conditions.

| Treatments | Conc. (%) | No. of released larvae | Mean % mortality | | | |
|-------------------------|-----------|------------------------|------------------|-------|-------|-------|
| | | | 24h | 48h | 72h | 96h |
| <i>Ricinus communis</i> | control | 20 | 0 | 2 | 2 | 4 |
| | 5 | 20 | 8.51 | 8.89 | 9.07 | 9.50 |
| | 10 | 20 | 8.72 | 8.99 | 9.38 | 14.54 |
| | 15 | 20 | 22 | 25.62 | 34.05 | 36.12 |
| <i>Citrus paradise</i> | control | 20 | 2 | 4 | 4 | 6 |
| | 5 | 20 | 6.59 | 8.98 | 11.90 | 18.89 |
| | 10 | 20 | 16.27 | 18.99 | 21.80 | 22.20 |
| | 15 | 20 | 19.09 | 20.56 | 25.87 | 29.50 |

Table 1; Both the plant oils shows great effect on mortality of red flour beetle (*R. communis* and *Citrus paradise*) but it is shown that highest mortality was recorded by *R. communis* plant oil after 96hrs that is 36.12%.

Table 2: Percent mortality (Mean) of *T.castaneum* adults treated with green synthesized silver nanoparticles under store conditions.

| Treatments | Conc. (ppm) | No. of released Larvae | Mean % mortality | | | |
|-------------------------------|-------------|------------------------|------------------|-------|-------|-------|
| | | | 24h | 48h | 72h | 96h |
| <i>Ricinus communis</i> AgNPs | Control | 20 | 0 | 2 | 0 | 2 |
| | 100 | 20 | 18.87 | 19.0 | 22.12 | 25.76 |
| | 200 | 20 | 27.8 | 28.7 | 31.77 | 32.45 |
| | 300 | 20 | 32.12 | 33.9 | 36.61 | 41.40 |
| <i>Citrus paradise</i> AgNPs | Control | 20 | 0 | 0 | 2 | 2 |
| | 100 | 20 | 18.11 | 20.2 | 22.43 | 23.04 |
| | 200 | 20 | 29.92 | 31.98 | 32.06 | 32.98 |
| | 300 | 20 | 26.12 | 28.3 | 34.76 | 38.81 |

Table 2; Both the green synthesized silver nanoparticles shows effect on mortality of red flour beetle (*R. communis* AgNPs and *Citrus paradise* AgNPs) but it is shown that highest mortality was

recorded by *R. communis* AgNPs after 96hrs that is 41.40%.

Data for repellency

Table 3; Comparison of the mean percentage repellency of *T. castaneum* after exposure to various concentration of plant oil *R. communis* after 24hrs

| Concentration (%) | % Repellency \pm SE |
|-------------------|-----------------------|
| 5% | 41.60 \pm 2.98 c |
| 10% | 51.21 \pm 2.49 b |
| 15% | 54.36 \pm 2.33a |

The outcome of the repellency bioassays in table 3 revealed that repellency was found increased with increase in concentration for all extract of plants.

Maximum repellency 54.36% was recorded at 15% concentration while minimum 41.60% was recorded at 5% concentration.

Table 4; Comparison of the mean percentage repellency of *T. castaneum* after exposure to various concentration of plant oil *Citrus paradise* after 24hrs

| Concentration (%) | % Repellency \pm SE |
|-------------------|-----------------------|
| 5% | 28.60 \pm 2.98 c |
| 10% | 32.21 \pm 2.49 b |
| 15% | 36.36 \pm 2.33a |

The outcome of the repellency bioassays in table 4 revealed that repellency was found increased with increase in concentration for all extract of plants.

Maximum repellency 36.36% was recorded at 15% concentration while minimum 28.60% was recorded at 5% concentration.

Table 5; Comparison of the mean percentage repellency of *T. castaneum* after exposure to various concentration of *R. communis* AgNPs after 24hrs

| Concentration (ppm) | % Repellency \pm SE |
|---------------------|-----------------------|
| 100 | 49.60 \pm 2.98 c |
| 200 | 56.21 \pm 2.49 b |
| 300 | 67.89 \pm 2.33a |

The outcome of the repellency bioassays in table 5 revealed that repellency was found increased with increase in concentration for all extract of plants.

Maximum repellency 67.36ppm was recorded at 15% concentration while minimum 49.60% was recorded at 5ppm concentration.

Table 6; Comparison of the mean percentage repellency of *T. castaneum* after exposure to various concentration of *Citrus paradise* AgNPs after 24hrs

| Concentration (ppm) | % Repellency \pm SE |
|---------------------|-----------------------|
| 100 | 36.60 \pm 2.98 c |
| 200 | 42.21 \pm 2.49 b |
| 300 | 48.31 \pm 2.33a |

The outcome of the repellency bioassay in table 6 revealed that repellency was found increased with increase in concentration for all extract of plants. Maximum repellency 48.36 300ppm was recorded at 15% concentration while minimum 36.60% was recorded at 100ppm concentration.

Conclusion;

We used two plant oils (*R. communis* and *Citrus paradise*) against stored grain insect pest red flour beetle and also use green synthesized nanoparticles from these plants. All the treatments shows good results but the effect of *R. communis* AgNPs was more then the *Citrus paradise* AgNPs and also as compared to plant oils of these two plants. Hence the green synthesized nanoparticles of *R. communis* AgNPs is more reliable to control the *Tribolium Castaneum* then the *Citrus paradise* AgNPs. Repellency bioassay was done by area preference method. Silver nanoparticles repellency highest range was 67.89% at 15% concentration of *R. communis* and lowest was 48.31% at same concentration of the *C. paradise* oils, used. Hence, plant based insecticides can be helpful for the management of stored commodities insect pests.

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