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Research Article

Evaluation of Toxicity of Nanoparticles against *Bactrocera zonata* as Bio-Control agent

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ABSTRACT

Mango is economically important fruit. Pakistan occupies 4th rank in mango production and export. Due to insects attack, US$200 million annual losses occurs. Among all pests fruit fly is one of major problem of Pakistan. These are usually controlled by the use of highly toxic chemicals that cause hazardous effects on the environment and non target organisms. In current study, better ways for the control of fruit fly were evaluated. New technique metal nanoparticles are being used for control of *Bactrocera zonata*, to minimize the adverse effects of insecticides on living health. For this purpose, adults of *B. zonata* were collected from infested fruits in mango orchards at Multan. SNPs were biosynthetically prepared with specific protocol by lemon plant leaves and toxicity was evaluated against flies to check mortality at different levels of SNPs concentrations (0pp, 20ppm, 40ppm, 80ppm, 160ppm). Mortality was observed upto 7 days. Maximum mortality was observed at 160ppm (higher concentration) while minimum mortality was observed at low concentration (20ppm). It showed higher concentration appeared to be more effective for the purpose of control. Hence overall 70-80% mortality is obtained thus resulting as effective bio-control with less hazardous effects to environment & livings.

KEYWORDS: B. zonata, Bio-control, Siver nanoparticles, Eco-friendly, Green synthesis

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Introduction

Pests are considered as a major hurdle in the way of better agricultural production (Altieri, 2018). Major part of the world's food production is destroyed by the attack of diseases, insects and weeds (Pimentel *et al.*, 2002; Damalas, 2016; Dababat *et al.*, 2018). According to an estimation insects are the main cause of damage and economical loss in crop production worldwide (Kumar and Kalita, 2017).

Serious damage to mangoes occurs by insect pests (more than 492 species) and mites (26 species) including hoppers, inflorescence midge, borers, mealy bug, fruit fly and many more (Anon, 2016). Fruit flies are major problem of horticultural crops (Clarke, 2005). Fruit flies are the main cause of economic loss such as both quantitative and qualitative losses to fruits to almost all the horticultural crops present around the world (Ekesi and Billah, 2007).

Estimated number of widely distributed species of genus *Bactrocera* are almost four hundred which are found in different regions of Asia, Africa, Australia and also Pacific region (Drew *et al.*, 1994). The prominent species found in Pakistan are *B. zonata, B. dorsalis, B. cucurbitae, (Manzoor et al., 2017).* 30-80% horticultural crops damage is occurred by fruit flies (Mwatawala *et al.*,

2006) & annual loss is being estimated as $200 million in Pakistan (Anon, 2017)

The damage caused by fruit flies exceed more than 80% and increasing by each coming year, even 100% damage is estimated in some mango growing areas where no precautionary measures were applied to control the insect pest (Duyck *et al*., 2006; Khosravi and Shafaghi, 2013). So, the main aim of all control programe is to eradicate and minimize the activity of fruit flies (Vargas *et al*., 2010; Estes *et al*., 2012).

B. zonata has a wide host range, it causes severe infestation in many fruits like; peach, guava, apricot (EPPO, 2005; Ghanim, 2009). Now it increased its host range and effects the commercial crops like mango, citrus, apple and many more (Samea and Fetoh, 2006). Pakistan's export is under a great threat by European Union (EU) due to attack of fruit fly on agricultural produce especially mangoes. EU already rejected more than 200 consignments of fruits infested by fruit fly. So, it resulted that EU gave yellow card to Pakistan for the export of mangoes and warned five more shipments of fruit fly infested mangoes will be the end of Pakistan's export to European countries (EU, 2104).

For the control and betterment of crops, pesticides are being used. Insecticides contribute 2nd biggest portion among all pesticides (5.2 billion pounds) being used world widely. Pesticides usage is the easiest way to control the pests (insects) but use of chemicals have dangerous outcomes. WHO and UN environment program estimated almost 3 billion agriculture workers suffers from server chemical poisoning which leads to death of 18000 (Miller, 2007).

Insecticides also effects the Environment, cause pollution, threats the living species, one of major cause of destruction of habitats (Palmer *et al*., 2007) which leads to the reduction in biodiversity and losses of endangered species, effects nitrogen fixation (Lin *et al*., 2013) also declines pollinators (Goulson, 2013). Use of metal nanoparticles (NPs) is a new innovation which was first recommended by lacey and lacey (1990) to use instead of pesticides, also having broad variability of metabolites (Awwad and Salem, 2012).

Nanotechnology has revolutionized the world with tremendous advancements in many fields of science like engineering, biotechnology, analytical chemistry and agriculture. Their use in crop protection is just in its infancy2. Nanomaterials measure between approximately 1 and
100 nm. "Nano" is a Greek word which means "dwarf" 10-9, or one billionth of something. Nanoparticles are very small sized substances having less than 100nm size (Laurent *et al.*, 2010) and depending upon physical appearance of these particles, they can be in various dimensions such as 0D, 1D, 2D or 3D (Tiwari *et al.*, 2012). While depending upon various size and shape the nanoparticles shows characteristics colours such as black and dark black, wine red, purplish colours respectively (Dreaden *et al.*, 2012). Although the nanoparticles are smaller in size but complex molecules consisting of three main layers (i) surface layer (metal ions, polymers, surfactants) (ii) the shell layer (iii) the core, it is the center most part of the nanoparticles (Shin et *al.*, 2016).

Nanoparticles synthesized from plant tisses have are of greater importance in managing insect pests (). Synthesis of nano-particles using various metals such as silver, copper, iron, glod, aluminium, pladium and titanium have attained great importance in the field of nano-technology (Sekhon, 2014). Among all, the best metal is silver, due to its amazing properties researcher prefer silver nanoparticles, because of its usage in various disciplines of science including Agriculture, physics, biotechnology, medicine, chemistry, physics, and mechanical engineering (Samarrai, 2012).

In recent studies among metallic nano-particles, silver is one of most synthesized nanoparticles with almost 500 tons/year production of nano-material (Larue *et al.*, 2014). Silver nanoparticles can be synthesized by using different techniques such as sol gel, ion sputtering, chemical reduction, physical reduction and by using electrodes and many more (Padalia *et al.*, 2014; Bindhu and Umadevi 2015; Mahdi *et al.*, 2015; Sre *et al.*, 2015). All of these

methods for the synthesis of nano-particles involves hazardous chemicals which not only affect our environment but also living organisms (Ahmed *et al.,* 2015).

Previously different methods such as chemical and physical ways were used to synthesize the nanoparticles but these appear to be responsible for hazardous to environment due to the use of toxic chemicals which are also cost expensive (Ahmed *et al.*, 2016; Baruwati *et al.,* 2009; Ahmed and Akram, 2015; Lin *et al.,* 2013). So, a new and recent alternate way evolved for the synthesis is known as green synthesis also known as" green revolution" in the fied of nanotechnology. Green synthesis involves the use of plant extract, sugars, micro-organisms as capping agents and reactants are proved attractants in the field of nano-technology (Kharissova *et al.,* 2013; Ahmed *et al.,* 2014; Ahmed and Ikram 2015).

This study was about the use of nanoparticles (NP) to determine the toxicity against *B. zonata* to the innovation of nanopesticides.

MATERIALS AND METHODS:

Collection & Rearing of Insects

Fruit flies were collected from different areas of region Multan. Adults of *B. zonata* were collected from installed traps of methyl eugenol and other attractants from the mango orchards of MNS- University of Agriculture, Multan.` Larvae were collected from the damaged and rotten fruits which were bought from the local market of Multan. For the purpose of rearing, rotten fruits and captured adults were brought to lab of MNSUA. Males and females both were collected from the damaged fruits and according to their day of emergence shifted to separate rearing cages and reared under lab conditions (25±2˚C and 60-75% R.H). The culture of *B. zonata* was established in cages (30×60×60 cm) whoes 2 sides were made of nylon mesh for the purpose of aeration. Artifical diet (Vayssières *et al.,* 2015) was provided to larvae when they emerge out within 3 days of eggs laying. Lavae stage lasts for 1-2 weeks and feed on artificial diet until it enclose itself in pupal case. Adults emerge within few days from pupal case. Adults were collected and shifted to new cage for mating and oviposition. 24 hours were given for mating and then fresh fruits were provided for the oviposition of *b.zonata*. Eggs were collected from that fruits and shifted to petri dishes for their hatching and all this life cycle continues and reared in lab of MNSUA, Multan .

Table: Artificial diet

Sr. No	Ingredients	Quantity
1	Egg	1 egg
2	Honey	2 tps
3	Vitamin B	1 tea spoon
4	Banana	2fingers

Biosynthesis of Nano-Particles:

Materials

Bio-synthesis of silver nano-particles (SNPs) was done by using fresh green leaves of lemon (*C. limon*) and aqueous solution of $AgNO_3$. Fresh green leaves of lemon were collected from the fields of MNS-University of Agriculture, Multan. To prepare metallic nanoparticles of silver, chemical named Silver nitrate was used. Distilled water and ethanol was required to wash all the equipments used for the purpose of preparation of SNPs. All solutions were prepared in deionized water

Preparation of Leaves extract

Leaves extract of lemon leaves was prepared which were collected from the fields of MNS-UAM, Multan. Leaves were thoroughly washed by using distilled water to remove the

dust particles and then spreaded on paper for the purpose of sun drying. After drying of leaves, 10g leaves were weighted and dissolved in 100ml of deionized water. Beaker was placed in hot water bath and heated for 15-20mins at temperature of 90˚C.

Extract colour was changed from yellowish to green. After 20mins, extract was removed from hot water bath and let it cooled at room temperature. Then extract was filtered through filter funnel by using Whatman filter paper No.1. leaves extract was used in the preparation of SNPs and stored for further use in synthesis procedure.

Preparation of Silver nitrate solution

1mM aqueous solution of silver nitrate was prepared by dissolving the silver nitrate in 100ml of deionized water. Molecular mass of silver nitrate is 169. To prepare the 1mM solution in 100ml, 0.169 was dissolved in 100ml of deionized water. $AgNO_3$ was properly stirred in solution to avoid any precipitation.

Synthesis of Silver Nano-particles

10ml of leaves extract was dissolved in 100ml of 1mM aqueous solution of $AgNO_3$. For proper dilution of leaves extract in aqueous solution of AgNO3, the solution was heated on hot plate for 15-20mins at temperature of 90˚C until the fumes started to evaporate from the solution. The colour of extract changed from orange to brown and then brown to dark brown. The change of colour indicated the synthesis of SNP's. The solution was cooled and kept for overnight at room temperature. Synthesized SNP's were stored at -4˚C for further use in experiments.

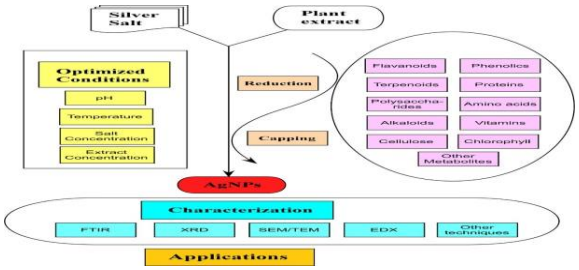


Fig: Flow chart for synthesis of nanoparticles

Characterization

Characterization was done by Ultraviolet-visible Spectroscopy (UV-VIS), Dynamic light Scattering (DLS) and Transmission Electron Microscopy (TEM), Zeta sizer, and SEM. Evaluation of insecticidal properties of nano-particles

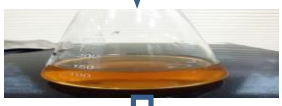
To check the efficacy of nanoparticles as a bio-control of fruit fly, different concentrations (20, 40, 80, 160ppm) of silver nanoparticles were prepared. Petri dishes containing artificial diet was treated with each concentration with 3 replications, along with control treatment with water. Trail was conducted in plastic jars, tightly covered with myuslin cloth under optimum laboratory conditions (25˚C temperature, 60% R.H) of MNSUA, Multan.

Mortility was recorded after daily continuously upto 7 days. Statistical Analysis Recorded data of all experimental studies of fruit flies was analyzed by using

Fig: Change in Colour

After mixing leaves extract an AgNO solution, pal yellow color appears

Within 10-15 mins color changes from pale yellow to orangish



Upon heating color changes into light brown

After cooling at room temperature finally dark brown color appears, as indication of nanoparticles,

Minitab v13.2 software (Minitab 2002 Software Inc., Northampton, MA, USA) to test the least significance followed by analysis of variance technique. Data (treated and untreated) was also compared by using

t-test (Tukey's Kramer test) at 5% significance level. Mortality of fruit flies by fungal infection and toxicity of nanoparticles was adjusted by using Abbott's (1925) formula.

RESULTS

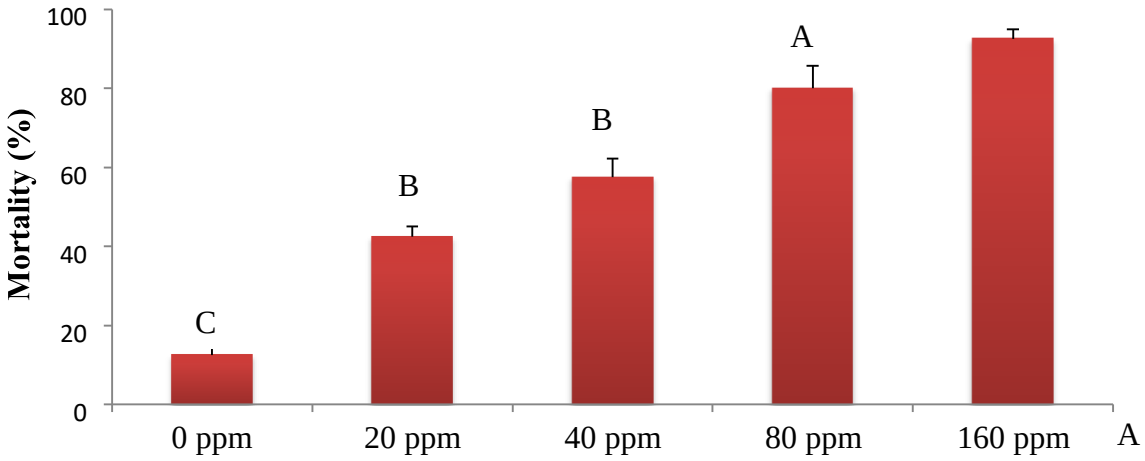
Effect of Silver nanoparticles against *B. zonata*

The experiment was conducted to check the toxicity of metallic (Silver) nanoparticles against *B. zonata*. Artificial diet was treated with bio-synthesized silver nanoparticles and fed *B. zonata* to check the mortality caused by nanoparticles in order to use as a biological control agent against fruit flies. Flies were kept in a cage and then mortality was checked for a week. All through the 7 days, mortality was recorded and compared it with the controlled ones (whose diet were free of any metallic nanoparticles)

The T1 shows the percent mortality due to effect of different concentrations (20, 40, 80, 160ppm) agamist *B. zonata* along with control parameter (without treated artificial diet). Maximum mortality upto 80% was showed under the influence of 160ppm concentration as well as minimum mortality appears at control other than that 20ppm concentration.

The T2 same as T1 shows the percent mortality of the *B. **zonata*** due to silver nanoparticles. It shows the days wise data upto 7 days. It shows the comparison between the days. Maximum mortality was recorded upto 7 days at maximum concentration of 160ppm of silver nanoparticles. It shows as the days increases the mortality ratio gradually increases with the increase of the days.

So, percent mortality is successfully obtained by using silver nanoparticles as a biological control agents and the obtained results were tested and checked by 5% level of significance.

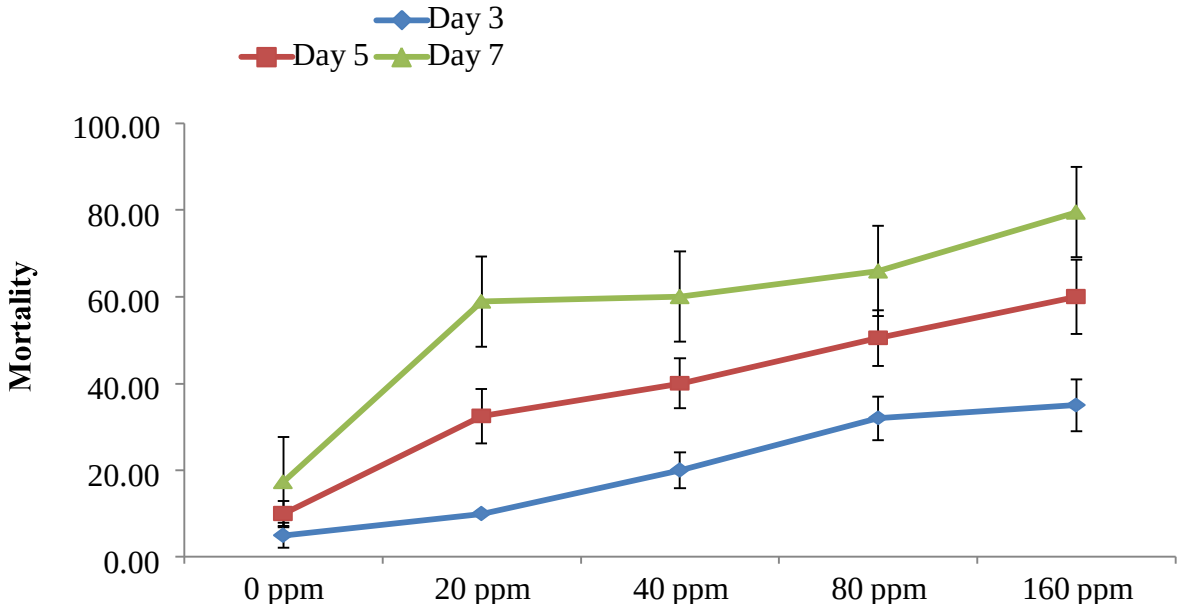


Concentration

Fig: Percent %mortality (mean±S.E) of *B, zonata* caused by different concentrations (20, 40,80 and 160ppm) of silver nanoparticles along with control (0ppm) flies.

Comparison of %mortality of *B. zonata* by Silver nanoparticles at different day intervals

Adults of *B. zonata* were treated with different concentration (0, 20, 40, 80 and 160ppm) and percent mortality was compared regularly at different day intervals upto 7 days.



Concentration

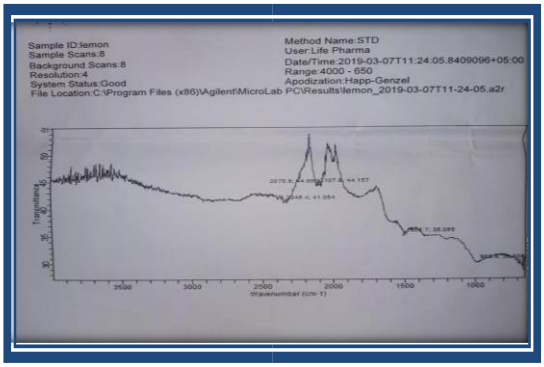


Fig: Comparison of %mortality between different days (D3, D5, D7) at different concentrations of nanoparticles (20, 40, 80 and 160ppm) along with control of 0ppm.

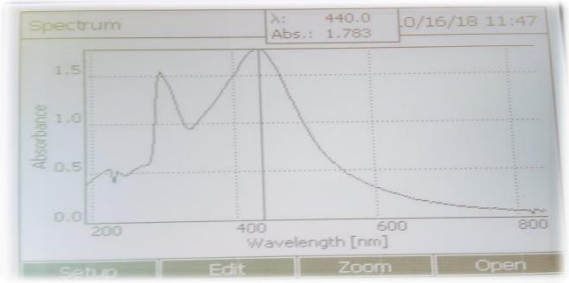


Fig: UV-visible light spectrometrography, the maximum wavelength was observed at 400nm with the maximum absorption power of 1.783 of lemon extract which easily does reduction of silver metal.

Charaterization

Fig: The sample of SNP's synthesized by lemon leaves extract was first diluted with di-ionized water and then sample was run which results in display of photographic image of the resolution scanning which indicates its range from 4000-650 with highest peak between 2500 – 2000 thus representing nano sized particles.

Discussion

Fruit flies are major problem of horticultural crops (Clarke, 2005). The prominent species found in Pakistan are *B. zonata, B. dorsalis, B. cucurbitae, (Manzoor. S et al., 2017).* Estimated number of widely distributed species of genus *Bactrocera* are almost four hundred which are found in different regions of Asia, Africa, Australia and also Pacific region (Drew *et al.*, 1994). 30-80% horticultural crops damage is occurred by fruit flies (Mwatawala *et al.*, 2006) & annual loss is being estimated as $200 million in Pakistan (Anon, 2017)

B. zonata has a wide host range, it cause severe infestation in many fruits like; Mango,

peach, guava, apricot (EPPO, 2005; Ghanim, 2009). Due to its severe infestation, mango orchards are being highly affected, especially affecting the export in other countries thus resulting in huge economic loss. Insecticides are mostly preferred for the control of fruit flies thus contaminate the fruits and also causes environmental pollution. To overcome the serious threat of fruit flies to fruits, biological control is being preferred, among which entomopathogenic fungus have great importance for the insect pest control, which is less destructive and less poisonous to health.

Same as use of entomopathogenic fungi, metallic nanoparticles (silver) were also synthesized biosynthetically, thus using plant leaves extract of lemon plant, the end product appears dark brown in color. Same as green synthesis of silver nanoparticles were reported by using the leaves extract of neem tree although, the endproduct have the same dark brown color of silver nanoparticles (Ahmed *et al.*, 2015). Green synthesis of silver nanoparticles by using *Lonicera japonica* leaf extract was reported by (Balan, 2015). This study was also to check the toxicity of silver nanoparticles against *B. bzonata* with different levels of concentrations (20, 40, 80 and 160ppm) thus resulting in more mortality as by the highest concentration of SNP's. Same as bioassay was conducted on rice weevil which resulted in having high mortality of rice weevil (Goswami, 2010), thus making it more preferable for the control of insect pests instead of use of insecticides which are environmental hazardous and also cause of health problems.

Conclusion

The present study represents that metallic nanoparticles are effective for the bio-control of *B. zonata.* Metallic nanoparticles is new and innovative way for the control of pests but it requires further more studies to evaluate its toxicity against *B. zonata*.

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