



EVALUATION OF NUTRITIVE VALUES AND MICROBIAL CONTENT OF WHEAT GRAINS TREATED WITH SOME TYPES OF NANO PARTICLES TO CONTROL *Sitophilus granaries* (L.)

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Rania S. Rashwan¹ and Abeer A. Abu-Zaid²

1- Plant Protection Dept., Fac. of Agric., Ain Shams Univ., Cairo, Egypt

2- Food Technology Research Inst., Agric. Res. Center, Giza, Egypt

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ABSTRACT

Nanotechnology has become promise field as a new approach for pest managing in recent years. Laboratory experiments were conducted to evaluate the toxicity of synthesized silver nanoparticles (AgNPs) by using aqueous plant extract (Rosmary, Laura and Cardamom) and their concentrations against main stored grain insect pest (*Sitophilus granarius*), microbial contents and nutritive value. Wheat grain which were treated with Rosmary 5 showed 100% mortality for adults of *S. granarius* after 120 h., while the same efficacy of Laura5 and Cardamom 5 recorded after 144 h. LC₅₀ and LC₉₀ values of the synthesized silver nanoparticles were identified against *S. granarius* indicated that the toxicity of AgNPs varied according to the plant extractio and exposure period. The carbohydrate, fiber, fat, protien and ash content of the treated wheat grains were significantly higher than control. While, the microbial contents were not detected after treatments by most AgNPs concentrations. In conclusion, the synthesis of AgNPs with high amounts from each plant extract and lower concentration from AgNo₃ (1 µg/ml) can be used as a valuable tool in pest management programs of *S. granarius*, reducing microbial content, and increasing the nutrition values, that led to increase shelf life of stored wheat grain. The results showed that these compounds could be used as a source of bioactive compounds safely for ecosystem.

INTRODUCTION

Wheat (*Triticum* spp.) is the most important food crop for more than half of the world's popula-

tion used for human food and livestock feeding. It is considered a good source of carbohydrates, B vitamins and minerals. Wheat grains have higher protein content than other cereal grains (Ileke and Bulus 2012). The grains can be contaminated by a great variety of bacteria, yeast and fungus during storage. These pathogens may affect the wheat resulting in a reduction of the grain quality; by changing the chemical composition or release of natural toxins that cause human and animal toxica-tion (Scudamore, 2005).

Losses during wheat grains storage are also due to infestation with insect pests that affect the quantity and quality of crop as well as germination of seed viability. Wheat is attacked by various insect pests during storage. The wheat weevil, *Sitophilus granarius* (Coleoptera: Curculionidae), is the most destructive insect pest of stored grains as well as stored products (Usha Rani and Rajasekharreddy, 2010). Both, the adults and larvae feed on whole grains, they attack, corn, oats, sorghum, beans and cereals. Currently, control of these pests is primarily dependent upon repeated application of synthetic insecticides (Hasan and Reichmuth, 2004).

There are many chemicals that are toxic to stored-grain pests, including insecticides such as organophosphates, pyrethroids and fumigants such as methyl bromide and phosphine (Park et al 2003 and Kljajic & Peric, 2006). These chemicals are effective for pest control, but have many problems to the environment. It has also undesirable effects, including toxicity to nontarget organisms and fostered environmental and human health concerns (Lee et al 2001).

Nanotechnology holds immense potential in the area of new pesticides. It represents a new generation of environmental remediation technologies that could provide cost-effective solution to some of the most challenging environmental cleanup

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problems (Chinnamuthu and Murugesu Boopathi, 2009). Silver is used in many applications in pure free metal or in compound form because it has antimicrobial activity against pathogens, it is also nontoxic to humans. Nitai et al (2010) proposed the application of surface functionalized silver nano particales as an insecticide to protect agricultural products. Reddy et al (2007) stated that there are many studies on the toxic effects of nanoparticles on bacteria, fungi, and animal pathogens.

Biological method of nanaoparticales synthesis using microbes and plant extracts is more important due to its eco-friendly, cost effective, flexible, main point is the evasion of toxic chemicals and safe for human therapeutic use .The spices are new face in the green synthesis and already Crozier et al (2006) reported the synthesis of silver nanoparticles using seeds of cardamom. Due to the presence of aromatic flavour compounds, the seeds of cardamom were likely to be responsible for the reduction of silver ions to silver nanoparticles. The Cardamom (*Elettaria cardamom*) venerated as “Queen of spices” belongs to Family, Zingiberaceae. Plants synthesize a vast range of secondary metabolities with a significant portion consisting of phenolic and flavonoid compounds. Jobitha et al (2012) photosynthesized Cardamom silver nanoparticles without use of any toxic chemical and showed antimicrobial activity against pathogens.

Rosmarinus officinalis L. (Family: Lamiaceae), commonly referred to as rosemary, belongs to mint family. Rosemary has a long list of claims pertaining to its medicinal usage including antibacterial, antioxidant, chemo preventive agent, anti-carcinogenic and useful as natural feed additives. Sulaiman et al (2013) synthesised of Ag nanoparticles, reducing the silver inos present in the solution of silver nitrate by aqueous extract of *R. officinalis* .These biologically synthesized nanoparticles were found highly toxic against different pathogenic.

Laurus nobilis, commonly known as bay leaves, belongs to Laureacea family. It is widely used as a spicy fragrance and flavor in traditional dishes. Laurel leaves and extracts are used to suppress high blood sugar, fungal and bacterial infections, and gastrointestinal problems. It also exhibits anti-inflammatory, and antioxidant properties. Infusions of dry bay leaves are used in folk medicine for their stomachic and also to treat gastric diseases (Dall'Acqua et al 2009). Khalil et al (2015) synthesized gold nanoparticles (AuNPs) using aqueous leaf extract *Laurus nobilis* and used

it to inhibit multidrug resistant Gram Positive bacteria (*Bacillus subtilis*).

So, this study aimed to investigate the mortality affected by synthesized AgNPs by each of the plant extract of Rosemary, Laura and Cardamom against *Sitophilus granarius*. Furthermore, determining the microbial content and nutrition value of wheat grains after treated by AgNPs .

MATERIALS AND METHODS

1. Insect rearing

Sitophilus granarius was collected from infested wheat obtained from a local market; it was reared in plastic jars under laboratory conditions of 27°C±1 and 65±5 % relative humidity (RH). Adults used in the experiment were almost of the same age (three days after emergence).

2. Biosynthesis of silver nanoparticles

Silver nanoparticles (AgNPs) and their concentrations were synthesized by each of the three plant species (Rosemary, Laura, and Cardamom) according to Abu-Zaid et al (2016^b). Adding different volumes (1, 3, 5, 7, 9 ml) of each plant extract and different volumes of distilled water (9, 7, 5, 3 and 1 ml, respectively) to 5 ml of 3 mM AgNO₃. The concentrations of AgNO₃ in each treatment were 8.6, 3, 2, 1.2 and 1 µg/ml respectively.

3. Insecticidal assay

Bioassay was performed on the adults of *Sitophilus granarius* in small plastic jars. Dimensions of each jar were 6x10 cm. The caps were perforated to allow aeration. A weight of twenty five grams of wheat was placed in each jar. Wheat grains were mixed with synthesized silver nanoparticles concentrations by using each plant extract of Rosemary, Laura and Cardamom, all Jars were shaken manually for approximately 1 minute to achieve equal distribution of nanoparticles on wheat (Subramanyam and Roesli 2000). There were five replicates for each concentration. For the control, wheat grains were left without mixing any nanoparticles. Ten adults of *S. granarius* were introduced for each replicate. All bioassay data were performed at 27±1°C and 65±5% RH. Insect mortality was checked after every 24 hours till the end of mortality of all individuals as mentioned by Debnath et al (2011).

4. Microbial assay

The total microbial content of infested wheat that treated with different concentrations of silver nanoparticles by using nutrients, MacConkey and Malt agar was estimated according to **Apha (1980)**. Initial suspensions were prepared by transferring one gm from each sample to 1% peptone water. Serial dilution (up to 10^6) was done in peptone water. After incubation, individual colonies were counted according to **ICMSF (1996)**. Three replicates were done for each concentration.

5. Determination of Proximate Composition of Dried Samples

Percentages of crude protein, fat, crude fiber, ash and total carbohydrates were determined after analysis of for the dried wheat grains that were treated with different concentrations of silver nanoparticles as described by the **AOAC (2005)**.

6. Statistical analysis

The mortalities data were analyzed with SAS program (**SAS Institute. 1988**) followed by one way ANOVA and Duncan's multiple range tests (**Duncan, 1955**) to compare mortalities among concentrations. The results were expressed as mean (\pm SD). Lethal concentrations (LC_{50} and LC_{90}) were estimated using probit analysis. All differences were considered at 5% probability level.

RESULTS AND DISCUSSION

1. Contact toxicity bioassay

Laboratory experiments were conducted to evaluate the pesticidal activity of five concentrations of synthesized silver nanoparticles (AgNPs) using extract of three plant species (Rosemary, Laura, and Cardamom) against adults of the wheat weevil *Sitophilus granarius*. Data in (**Table 1 and Fig. 1**) show the mortality percentages of *S.granarius* adults treated by different concentrations of synthesized silver nanoparticles (AgNPs). Data revealed that mortalities were not at all proper effect on adults, where it increased with the decreases of $AgNO_3$ concentration and increase of plant extract concentration. For rosemary, percent mortalities of insects treated with AgNPs started

after 48 hours under the laboratory conditions, the higher concentration of rosemary-1 (8.6 μ g/ml $AgNO_3$) recorded 70 % mortality after 144 hours while treated with Rosemary-5(1 μ g/ml $AgNO_3$) recorded 100% after 120 hours. For the application of synthesized AgNPs by using lura extract on adult stage, it began positive effect after 24 hours; for Laura-3, Laura-4 and Laura-5 (2, 1.2 and 11 μ g/ml $AgNO_3$ respectively); those showed 100 % mortality after 144 h. Treatment with AgNPs by using cardamom extract demonstrated the same results at cardamom -5 (1 μ g/ml $AgNO_3$) after the same period. Statistical analysis of data proved that there were highly significant differences represented by (F and L.S.D. values) between treatments with nanosilver particles using three plant extracts and its concentrations as shown in (**Table 1**).

These results agree with **Sankar and Abideen, (2015)**, who studied the pesticidal activity of synthesized silver nanoparticles and synthesized lead nanoparticles from mangrove extract against *Sitophylus oryzae* adults. This result showed that the synthesized nanoparticles using plant extract was more effective than the aqueous extract. **Abduz Zahir et al (2012)** showed the pesticidal activity of the synthesized AgNPs using *E. prostrata* extract against the adults of *Sitophylus oryzae* was more effective than the aqueous extract, that mean nano formulations are expected to have significant impacts on the fate of active ingredient. Management of stored-grain pests stands traditionally on use of synthetic insecticides and long term application of these chemicals develops resistance to pesticides. In recent years, nanoparticles have received much attention for controlling of pests in agriculture (**Elenka et al 2010**). **Stadler et al (2010)** applied successfully nano alumina against two stored grain pests. **Rouhani et al (2008)** showed that the both nanoparticles (silica and silver) were highly effective on adults and larvae of *Callosobruchus maculatus* with 100% and 83% mortality, respectively, so they can be used as a valuable tool in pest management programs of stored grain insect pests.

The probit statistics estimates of LC values of AgNPs were examined where data indicated that the toxicity of AgNPs varied according to the concentration and exposure period. Data in **Table (2)** showed the calculated LC_{50} and LC_{90} of AgNPs after 72, 96, 120 and 144 h. against adults of *Sitophilus granarius*. These results were highly similar with the findings of **Rouhani et al (2008)**.

Table 1. Adult mortalities (mean \pm SE) of *Sitophilus granarius* treated with synthesized silver nanoparticles by using three plant extracts

	Exposure period											
	24 h.		48 h.		72 h.		96 h.		120 h.		144 h.	
	Mean no. of dead adults \pm se	Mortality %	Mean no. of dead adults \pm se	Mortality %	Mean no. of dead adults \pm se	Mortality %	Mean no. of dead adults \pm se	Mortality %	Mean no. of dead adults \pm se	Mortality %	Mean no. of dead adults \pm se	Mortality %
Rosmary-1	0	0 b	1 \pm 0.2	10 c	4.5 \pm 1.2	45 c	5 \pm 0.13	50 c	6 \pm 0.41	60 c	7 \pm 0.32	70 d
Rosmary-2	0	0 b	4 \pm 0.32	40 b	4.5 \pm 0.3	45 c	6 \pm 0.5	60 b	6.5 \pm 0.3	65 c	8 \pm 0.4	80 c
Rosmary-3	0	0 b	4 \pm 0.25	40 b	4.5 \pm 0.036	45 c	6 \pm 0.6	60 b	6.5 \pm 0.2	65 c	8 \pm 0.2	80 b
Rosmary-4	0	0 b	4 \pm 0.1	40 b	5 \pm 0.21	50 b	6 \pm 0.51	60 b	7 \pm 0.3	70 b	9 \pm 0.3	90 b
Rosmary-5	2 \pm 0.2	20 a	5.5 \pm 0.6	55 a	8.5 \pm 0.6	85 a	9 \pm 0.3	90 a	10	100 a	10	100 a
Control	0	0 b	0	0 d	0	0 d	0	0 d	0	0 d	0	0 e
L.S.D.	-	1.14	-	2.5	-	2.14	-	2.08	-	1.7	-	2.8
F value	-	152.3	-	218	-	494	-	627	-	627	-	321
Laura-1	0	0 d	2.5 \pm 0.6	25 c	3 \pm 0.6	30 d	3.5 \pm 0.31	35 c	5.5 \pm 0.25	55 c	6.5 \pm 0.4	65 b
Laura-2	0.5 \pm 0.1	5 c	2.5 \pm 0.54	25 c	3.5 \pm 0.23	35 d	3.5 \pm 0.6	35 c	5.5 \pm 0.3	55 c	6.5 \pm 0.6	65 b
Laura-3	1.5 \pm 0.21	15 b	2.5 \pm 0.32	25 c	5 \pm 0.40	50 c	4 \pm 0.52	40 c	6 \pm 0.3	60 b	10	100 a
Laura-4	2.5 \pm 0.13	25 a	4.5 \pm 0.32	45 b	6 \pm 0.3	60 b	6.4 \pm 0.23	64 b	6.5 \pm 0.14	65 b	10	100 a
Laura-5	2.5 \pm 0.21	25 a	6 \pm 0.32	60 a	7 \pm 0.23	70 a	7 \pm 0.32	70 a	8 \pm 0.42	80 a	10	100 a
Control	0	0 d	0	0 d	0	0 e	0	0 d	0	0 d	0	0 c
L.S.D.	-	3.2	-	10.4	-	2.5	-	2.4	-	2.2	-	4.2
F value	-	403	-	136	-	302	-	261	-	516	-	261
Cardamom-1	0.5 \pm 0.1	5 b	1.5 \pm 0.6	15 b	2 \pm 0.3	20 c	3 \pm 0.3	30 b	6 \pm 0.3	60 b	6.5 \pm 0.23	65 c
Cardamom-4	0.5 \pm 0.1	5 b	1.5 \pm 0.21	15 b	2.5 \pm 0.2	25 b	3.4 \pm 0.2	34 b	6.5 \pm 0.3	65 a	7 \pm 0.6	70 b
Cardamom-5	1 \pm 0.1	10 a	2 \pm 0.3	20 a	3 \pm 0.3	30 a	4 \pm 0.5	40 a	7 \pm 0.3	70 a	10	100 a
Control	0	0 c	0	0 b	0	0 d	0	0 c	0	0 c	0	0 d
L.S.D.	-	2.4	-	4.1	-	4.3	-	4.7	-	4.01	-	4.01
F value	-	80	-	162	-	316	-	236	-	326	-	265

*Means within a column within treatments followed by different letters are significantly different ($P \leq 0.05$; using Duncan's multiple ranges clarifying by LSD test)

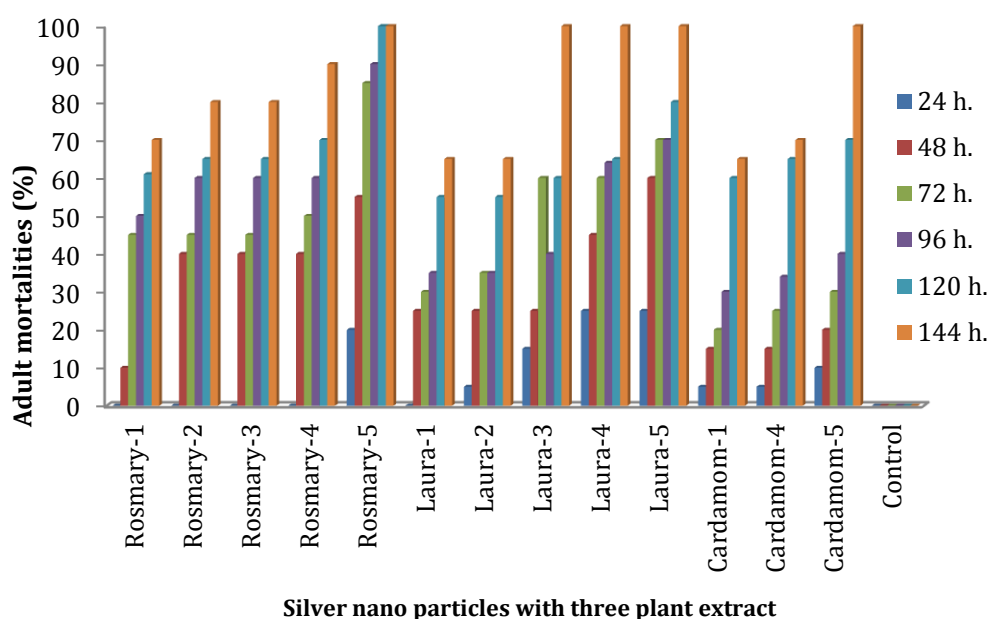


Fig. 1. Mortality percentages of *Sitophilus granarius* adults treated with synthesized nanoparticles with three plant extracts

Table 2. The LC₅₀ and LC₉₀ values (µg/ml) for silver nanoparticules using three plant extract against *Sitophilus granarius*.

Plant extract	LC values	exposure periods			
		72 h.	96 h.	120 h.	144 h.
Rosmary	LC50	1.1	7.79	10.8	14.36
	LC90	11.61	1.2	1.4	1.13
Laura	LC50	2.92	1.96	9.6	10.84
	LC90	6.404	1.9	1.26	2.30
Cardamom	LC50	-	12.5	12.4	13.9
	LC90	-	4.7	0.7	0.64

when calculating LC values of adults and larvae of *Callosobruchus maculatus* that treated with AgNPs.

1- Determination of nutrition values

Data in **Table (3)** recorded the proximate composition of the wheat samples which were treated with AgNPs by using three plant extracts (Rosmary, Laura and Cardamom). Statistical analysis showed significant differences at (P≤0.05) between the synthesized silver nanoparticules and its concentrations. Data illustrated that the lowest concentration of AgNO₃ and highest concentration of plant extract led to highest percentages of five

nutrition values; crude protein, fat, crude fiber, ash and total carbohydrates in treated wheat samples and vice versa. Plant extracts causes increasing in the bio components in treated wheat. **Jasim et al (2007)** indicated that treatment by garlic and liquorous on cucumber caused significant effect in carbohydrate content. It was noticed that carbohydrate contents were highest in treated grains compared to untreated. **(Jhanzab et al 2015)** AgNPs, significantly, enhanced most of the growth and yield attributes and nutrient use efficiency of wheat. Silver nanoparticles in low concentration caused significant improvement in maximum leaf area and highest grain yield while high concentration resulted in decrease in grain yield; so silver nanoparticles have stimulatory as well as inhibitory effect on wheat growth and yield. **Hojjat and Holjjat (2015)** investigated the effects of the concentrations of silver nanoparticles on plant growth parameters such as root length, fresh weight, dry weight, and % germination of Fenugreek (*Trigonella foenum-graecum*). Seed germination results indicated that AgNPs at their lower concentrations promoted seed germination and early seedling growth in Fenugreek, however at higher concentrations showed slight adverse effects. Additionally, the lowest amount of these parameters was found with control plants.

Table 3. Proximate composition of the treated wheat samples (on dry weight basis).

Samples	% OF COMPONENT				
	Crude protein	Fat	Crude fiber	Ash	Total Carbohydrates
Rosmary-1	9.71 ^e	1.75 ^d	2.27 ^d	1.32 ^c	70.33 ^d
Rosmary-2	10.26 ^d	1.82 ^{c,d}	2.46 ^c	1.48 ^b	73.50 ^c
Rosmary-3	10.57 ^c	2.00 ^c	2.52 ^{b,c}	1.50 ^b	76.30 ^b
Rosmary-4	10.87 ^b	2.16 ^b	2.63 ^b	1.65 ^b	76.39 ^b
Rosmary-5	12.33 ^a	2.37 ^a	2.97 ^a	1.90 ^a	79.45 ^a
Control	8.1	1.4	1.16	1.04	67.4
L.S.D.	0.230	0.165	0.11	0.176	0.234
F value	921	98	891.2	90.6	886.6
Laura-1	9.82 ^e	1.79 ^c	2.31 ^d	1.32 ^d	70.91 ^e
Laura-2	10.36 ^d	1.96 ^b	2.43 ^{c,d}	1.45 ^{d,e}	71.87 ^d
Laura-3	11.24 ^c	2.03 ^b	2.48 ^{b,c}	1.56 ^{b,c}	75.29 ^c
Laura-4	11.85 ^b	2.07 ^b	2.55 ^b	1.73 ^b	78.63 ^b
Laura-5	12.18 ^a	2.18 ^a	2.75 ^a	1.95 ^a	79.83 ^a
Control	8.1	1.4	1.16	1.04	67.4
L.S.D.	0.232	0.11	0.121	0.174	0.165
F value	826	118.3	7.6	107.9	180.6
Cardamom-1	10.46 ^c	1.97 ^b	2.19 ^b	1.18 ^{b,c}	71.11 ^b
Cardamom-4	12.07 ^b	2.02 ^b	2.32 ^{b,c}	1.27 ^b	77.15 ^c
Cardamom-5	12.90 ^a	2.22 ^a	2.54 ^a	1.67 ^a	80.26 ^a
Control	8.1	1.4	1.16	1.04	67.4
L.S.D.	0.323	0.146	0.146	0.217	0.146
F value	236	125.6	594	53.3	394

* Different letters within each column in the same treatment indicates significant difference.

2- Microbial contents in treated wheat grain

The bacteria and fungi species that arise commonly on stored wheat grains are generally non-pathogenic, though contamination with pathogenic bacteria such as *Salmonella*, *Escherichia coli*, *Bacillus cereus* and fungi may occur. The highest counts of aerobic bacteria were recorded in the control samples, which treated by plant extract only (Table 4). Enteric bacteria and fungi counts were of lower numbers in the control. *E. coli* is enteric bacteria, and their presence on stored grains is regularly an indication that birds or rodents have contaminated it. This may occur during harvesting, poor hygiene in road or rail trucks during transportation or poor pest control during storage.

Photosynthesized silver nanoparticles by plant extracts and their concentrations showed highest antimicrobial activity against the microbial contents as shown in Table 4. Bacteria and Fungi were not detected in wheat grain samples with all treatments except, Rosmary-1, Rosmary-2, Laura-1 and Cardamom-1. Abu-Zaid et al (2016) reported that

the biosynthesis of cardamom silver nano particles possessed highly significant increase of anti-bacterial against *K. pneumnonia* as compared to aqueous extract of cardamom. They also concluded that, the biosynthesized nanosilver plants were more effective than Laura aqueous extract. They attributed their results to the abundance of bioactive compounds in plant extract. These compounds were more effective when synthesis with AgNo₃ and converted to nano particles. These results agree with findings of Nanasombat and Lohasupthawee (2005) who used oil cardamom extract and scored lower inhibition zone, about 10 mm against *Klebsiella* sp. On contrary; Mishra and Behal (2010) scored highest degree of inhibition zone by cardamom aqueous extract (21mm) against *E.coli*.

Table 4. Results of microbial content (Aerobic and Enteric bacteria and Fungi counts) in the treated wheat samples

Name of sample	Aerobic bacteria	Coliform Fungi	
Rosmary-1	12	7	13
Rosmary-2	0.70	NG	1.2
Rosmary-3	NG	NG	NG
Rosmary-4	NG	NG	NG
Rosmary-5	NG	NG	NG
Laura-1	2.4	2	5
Laura-2	NG	NG	NG
Laura-3	NG	NG	NG
Laura-4	NG	NG	NG
Laura-5	NG	NG	NG
Cardamom-1	20	10	15
Cardamom-4	NG	NG	NG
Cardamom-5	NG	NG	NG
Control	184.4	48.4	56.4

Cfu/g: colony forming units per gram; NG: no growth detected

The correlation between biosynthesized cardamom silver nanoparticles and viability of VERO cell line was negative and highly significant, where "r" value = -0.9029, where at 3.125µg/ml concentration, AgNo₃ showed 100% viability of VERO cell line. At the same concentrations, the biosynthesis Rosmary and Laura silver nanoparticles were scored of lower cytotoxicity effect against normal cell, whereas all VERO cell line was viable 89.7 and 97.04% respectively. This suggests that silver nano particles from plant extract have great potential to act a safe alternative to antibiotics to fight the challenges of drug resistance concluded by Abu-Zaid et al (2016). In an earlier study by Sulaiman et al (2013) also inhibited 80% from HL-60 cell line by synthesized silver nanoparticles (1mM AgNps) at the similar incubation time.

CONCLUSION

The incorporation of synthesis silver nanoparticles to wheat grains improved the nutritional values of treated wheat and caused reduction in the population densities of *S. granarius* as main destructive insect pest where treatment with silver nanoparticles caused high mortalities among adults in few days. It also inhibited the microbial content in stored grains. Bacteria and molds common in the environment contaminated the grains and led to its spoilage after 6–8 days; however, the experiments ended after 6 days and the results of tests indicated that the grains become safe to use by consumer. Bacteria and fungi were not detected in some of the wheat grains. These are within the limit set by the Standard Organization of Egypt, which states that the counts of aerobic bacteria must not exceed 100 cfu/g and coliform growth must not be detected in stored wheat grains. The cost of green synthesis nanoparticles could be less. Whereas this method can be used safe in preserving food, therefore, Ag NPs has an excellent potential pesticide as stored grain as well as seed protecting agent if applied with proper safety trials. This study could lead to open up pathways of using nanomaterial based technology in integrated pest management.

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