Study Of The Effect Of Silver And Zinc Nanoparticles Prepared Biologically By The Fungus Beauveria Bassiana On The Feeding Behavior And The Insect Body Content Of Some Materials And Nutrients In The Periplaneta Americana L.

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KEYWORDS

Periplaneta americana L., Sodium element, Potassium element, Proteins, phosphorus element, Nanomaterials. Beauveria bassiana..

ABSTRACT

The study was conducted in the laboratories of the Department of Prevention / College of Agriculture / Tikrit University during the years 2023-2024 to study the effect of the nanomaterial resulting from treating the fungus Beauveria bassiana with a solution of silver nitrate and a solution of zinc oxide as it affected the daily feeding rate of the last-age nymphs of the Periplaneta americana, as this rate was 0.015 g / insect on the second day of treatment with the nanomaterial prepared using silver nitrate, while it was 0.04 for the zinc oxide treatment. While it was 0.07 g / insect for the control treatment, and the nanomaterial affected the two solutions in addition to the control treatment in a fluctuating manner during seven days of feeding. Both silver nitrate and zinc oxide treatments and the control treatment recorded 0.007, 0.05, 0.085 gm/insect on the eighth day of treatment. The treatment also affected the percentage of each of the fats for the concentrations of 0.125, 0.250, 0.500 mg/L and the control treatment if it was 23.8, 23.9, 24 and 23.8 mg) respectively for the silver nitrate treatment, while it was 22.3, 20.6, 19.5, 23.8 for the zinc oxide treatment. While the protein weight was 234.24, 127.64, 35.72, 382.81 mg/g for silver nitrate, while it was 304.37, 43.756, 233.125, 43.75 mg/g for zinc oxide. It also affected the potassium element, as the concentrations were recorded as 0.125, 0.250, 0.500 mg/L and the control treatment was 4.65, 2.36, 0.54, 6.22 mg respectively for silver nitrate and 5.14, 3.21, 0.75 and 6.25 mg respectively for zinc oxide. It also affected the weight of the sodium element, as the concentrations were recorded as 0.125, 0.250, 0.500 mg/L and the control treatment was 2.63, 1.22, 0.46, 3.24 mg respectively for silver nitrate and 2.45, 1.08, 0.42 and 3.34 mg respectively for zinc oxide. It affected the percentage of phosphorus element, as the above concentrations were recorded, 4.32, 2.61, 0.40, 6.13 mg for silver nitrate, while they were 5.12, 3.43, 0.70, 6.24 mg for zinc oxide.

1. Introduction

Most insects have nutritional requirements that vary in importance, such as proteins, carbohydrates, fats, vitamins, minerals, various elements, and water. These chemical compounds can be manufactured by the insects themselves, or obtained from food. The change in the diet of insects leads to a significant change in the proportion of nutrients acquired in the food, between species and growth stages of the same species, as a result of adaptation to specific environments where the intake of nutrients is limited to the types and diversity of available foods (Zai et al., 2018). Plant-eating animals, of which insects constitute the majority, feed on plants and are typically specialized in terms of nutrition, meaning that they consume only one or a few plant species. In contrast, carnivorous insects, which eat detritus, tend to be more generalist in terms of their nutritional system (Judith and Rana, 2016), and insects strive to achieve nutritional balance by carefully regulating their intake of many nutrients at a time, either from artificial diets or from natural foods. For insects, these substances typically include macronutrients (proteins, carbohydrates, and fats) and micronutrients (vitamins, minerals, and water), all of which are directly involved in physiological functions (Subhan & Minglin, 2023). Proteins are the most important nutrients required by the insect diet, and are the main source of nitrogen for the insect body, which is found in amino acids that may exist free in plants. However, they are most often linked together by peptide bonds to form proteins (Ksenija & Jutta, 2019). Proteins can be broken down into their basic components of amino acids and reshaped and converted into different proteins that can be used for a wide range of biological functions such as transport and storage. The process of protein formation in insects requires nine essential amino acids (arginine, pyridoxine, histidine, isoleucine, leucine, methionine, threonine, valine) (Victor et al., 2021). Insects that feed on smaller organisms have higher requirements for amino acids and lipids than for carbohydrates, reflecting the relatively high protein content and low carbohydrate content of animal tissues. However, insects that feed on plants generally require approximately equal amounts of amino acids and carbohydrates (Renske et al., 2017).

Lipids are an important source of energy, essential components of cell membranes, nutrient carriers, and defense compounds. Lipids consist of fatty acids, phospholipids, and sterols. They contribute to the synthesis of hormones (Papachova and Cahova, 2015). Insects can synthesize most fatty acids and phospholipids. To do this, unsaturated fatty acids are needed in the diet. Cholesterol is the major sterol, abundant in animal tissues but absent or present in low amounts in plants and fungal foods (Daniel et al., 2023). Insects also require minerals, which are inorganic nutrients, and are usually required in small amounts of less than 1 to 250 mg per day, as with vitamins and other essential nutrients, mineral requirements vary among insect species, for example phosphorus is an important component of adenosine triphosphate (ATP) and nucleic acid and is also essential for acid-base balance, and elements such as calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium and zinc are involved in the synthesis of coenzymes and metalloenzymes (Ajai et al., 2013). Sodium and zinc are essential in small amounts for many insects that require sodium, potassium and chlorine for their importance in maintaining osmotic balance between cells and intracellular fluid. Potassium, chlorine, calcium and sodium are essential for the excitability of tissues such as muscle cells and neurons, and potassium and magnesium are major players in bioenergetic activity, respectively, via the ATP pathway and glycolysis (Sargenti et al., 2017). Contaminated metals, present in the environment and passively ingested by insects, can displace dietary minerals and produce toxins from cholesterol by removing alkyl groups from sterols (Ursula et al., 2019). Excessive intake of some minerals can disrupt the excretory balance and cause toxic side effects. Severe mineral deficiencies can also alter the delicate balance in the body (Afridi et al., 2018). The study of insect nutrient regulation made great strides forward at the end of the 20th century when Raubenheimer and Simpson (1993) introduced a unified theoretical framework for nutrition studies known as nutritional engineering. This takes into account the multiple interactions between mechanisms that regulate the intake of different classes of nutrients. The diet of insects is affected by a range of environmental factors, including nanomaterials that can be produced naturally in the environment by the biological activity of microorganisms such as bacteria and fungi. For example, silver nanoparticles synthesized using fungi affect the behavior of pathogen vectors such as insects, with low toxicity and good biocompatibility. These results open up prospects for future investigations related to the use of these materials in the fields of health and agriculture (Goswami et al., 2010).

The mechanism of biosynthesis of nanoparticles using fungi may be intracellular or extracellular. In the case of intracellular synthesis, the mineral precursor is added to the fungal culture medium and is absorbed into the biomass. Therefore, it is necessary to extract the nanoparticles after synthesis, and use chemical treatment, centrifugation and filtration to disrupt the biomass and release the nanoparticles. Given that a wide range of fungi have the potential to be used in synthesis, it is important to consider their individual properties and optimize the synthesis conditions (Ottoni, 2017).

This research includes studying the effect of the nanomaterial prepared biologically by the fungus Beauveria bassiana on the feeding behavior and percentage of some materials and mineral elements in the American cockroach insect after treating the food medium with the nanomaterial prepared biologically using the fungus, silver nitrate and zinc oxide. These are the percentage of fats, proteins, sodium, potassium and phosphorus.

2. Methodology

For the purpose of conducting the study, it was necessary to collect large numbers of *Periplaneta americana*.This process was carried out in several places such as sewer openings (manholes) and garbage collection places near homes, the health center and some departments such as schools spread in the Yathrib district of Salah al-Din Governorate. The traps available in local markets as well as locally made traps were relied upon (Mansor and Al-Mallah, 2017). The nanomaterial was also prepared by the fungus *Beauveria bassiana* after treatment with silver nitrate and zinc oxide. To prepare the silver nanoparticles, a solution of silver nitrate AgNo3 was first prepared at a concentration of 1 mm by dissolving 0.017 grams of silver nitrate in 100 ml of sterile distilled water, mixing well and storing in opaque bottles to prevent it from being affected by light until the time of use (Karbasian et al., 2008). To form nanoparticles biologically from the fungus *Beauveria bassiana* after the formation of the fungal mat and filter the fungal mass using sterile filter paper and wash well with sterile distilled water, then placed in a 500 ml conical flask containing 100 ml of sterile distilled water, the flask was incubated for 72 hours and the fungal mass was filtered again using sterile filter paper to ensure the removal of all components of the medium, then 10 g of the fungal mass was weighed and then placed in 100 ml of silver nitrate solution AgNO3 prepared at a concentration of 1 mM and placed in the incubator in completely dark conditions. After the period, we filter the biomass again using filter paper and then centrifuging at a rate of 6000 rpm for 10 minutes, taking 100 ml of the fungal biomass filtrate and adding 100 ml of silver nitrate solution AgNo3 at a concentration of 1 Mm, which was prepared previously, leaving 100 ml of the filtrate for comparison and other tests, all flasks were placed in the vibrating incubator at a speed of 100 rpm at a temperature of 25-26 m under dark conditions for 96 hours (Sara et al., 2022). To confirm the formation of silver nanoparticles (AgNPs) outside the cell using a liquid culture medium for the *fungus Beauveria bassiana*, the color change was evidence of the formation of AgNPs particles, and electron microscopy and X-ray diffraction were used to detect these particles.

Intracellular nanoparticles (ZnO-NPs) were produced by fungi and isolated biomass by culturing fungi in malt glucose-yeast broth (MGYP) consisting of yeast extract and malt extract 0.3% each, glucose 1%, peptone 0.5%. The fungi were cultured in a 250 ml Erlenmeyer flask containing 50 ml of liquid medium containing (KH2PO4, 1 g), (K2HPO4, 7 g), (MgSO47H2O, 4 g), (NH4)2SO4, 1 g), (yeast, 1 g), (glucose, 15 g). The inoculated medium was incubated at 28 ± 2 °C and 180 rpm for 5 days, after which the fungal biomass was separated using Whatman No. 1 filter paper and washed extensively with deionized water. The collected fungal biomass was transferred to 100 ml of deionized water in a 250 ml Erlenmeyer flask and further incubated at 140 rpm for 72 h, the fungal biomass was filtered again using Whatman No. 2 filter paper and the collected cell-free filtrate was subjected to ZnO nanoparticle biosynthesis. 10 mL of ZnSO4 salt was added to 10 mL filtrate of each fungus and the pH was adjusted to 6.5 and incubated in a centrifuge for 150 rpm at 32 °C for 72 h, in the dark condition. The precipitation of white precipitate at the bottom of the flask indicated the formation of nanoparticles (Mona et al., 2019).

3. Result and Discussion

First: The effect of nanomaterial on the feeding behavior of fourth instar nymphs of Periplaneta americana L.

The results (Figure 1) showed a clear effect on the feeding behavior of the fourth instar nymphs of the *Periplaneta americana*, as the nanomaterial resulting from the fungus treatment with silver nitrate had a significant effect, as the daily consumption rate was less than 0.02 g/insect during the second day of the treatment, while the daily consumption rate was less at 0.01 g/insect from the third to the eighth day of the treatment, then the consumption rate increased to 0.02 during the ninth day of the treatment. While the nanomaterial resulting from treating the fungus with zinc oxide had a lesser effect than nitrate, as the daily consumption rate was 0.04 g/insect per day, then decreased to less than 0.02 g/insect during the third day, then the daily consumption rate rose again to more than 0.04 g/insect during the fourth day, then decreased again to less than 0.02 g/insect during the sixth day, then increased again to 0.05 g/insect during the eighth day and decreased to 0.025 g/insect during the tenth day. While the control treatment recorded 0.07 g/insect during the second day and more than 0004 g/insect during the third day, then the consumption rate increased to 0.08 g/insect during the fourth day and decreased to

0.02 g/insect during the sixth day, then the consumption rate returned to visit 0.085 during the eighth day, then this rate decreased to 0.06 during the tenth day. As noted from Table (1), the nanomaterial prepared by the fungus *Beauveria bassiana* using silver nitrate did not record a significant difference in the percentage of fat in the body of the American cockroach, as the percentage of fat for the concentration of mg/l 0.500 for the nanomaterial was 24%, while the percentage of fat in the body of the insect in the control treatment was 23.8, while the concentration of 0.500 for the nanomaterial resulting from the treatment of the fungus with zinc oxide recorded a significant effect, as the percentage of fat was 19.5% compared to the control treatment 23.8%, while it recorded The concentrations of 0.125 and 0.250 are 23.3 and 20.6%, respectively.

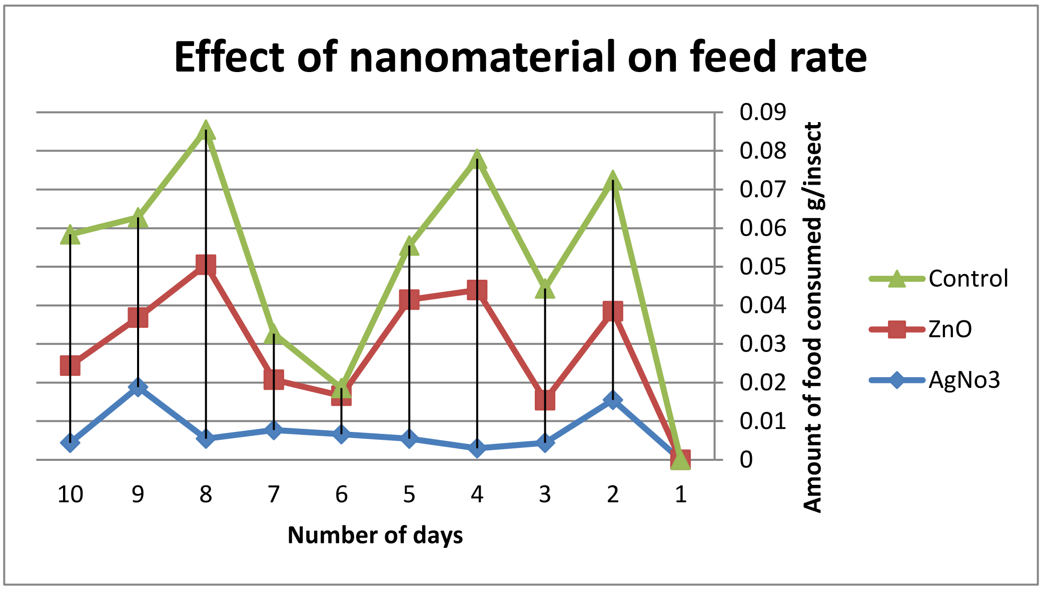

Figure (1) Effect of nano-solution of silver nitrate and zinc oxide for the fungi Beauvaria bassiana. added to the nutritional medium on the average daily food consumption of the fourth instar nymphs of the insect Periplaneta americana L.

Second: Effect of the nano-material on the amount of fat in the body of adult *Periplaneta americana*. The fourth instar nymphs were treated with the nano-material.

It is clear from Table No. (1) that the nano-material prepared biologically using the fungus *Beauveria bassiana* treated with silver nitrate solution did not significantly affect the amount of fat, as the concentrations recorded 0.125, 0.250, 0.500 mg / liter with a weight of 8. 23, 23.9, 24.0 respectively, while the control treatment recorded 23.8 mg / g. While the above concentrations of the nanomaterial prepared using zinc oxide solution were affected, as they recorded 22.3, 20.6, and 19.5 mg/g, respectively, while the treatment and control recorded 23.8 mg/g.

Table 1: Effect of different concentrations of the nanomaterial prepared biologically using the fungus Beauveria bassiana treated with silver nitrate and zinc oxide on the amount of fat in the insect Periplaneta Americana L

Materials used / Concentration mg/l	Fat weight mg/g		Arithmetic mean	Variance	covariance coefficient	Standard error of the mean	Standard deviation
	silver nitrate	Zinc oxide					
0.125	* 23.8	*22.3	23.05	1.64	0.06	0.52	1.281

0.250	23.9	20.6	22.25	4.30	0.09	0.85	2.074
0.500	24.0	19.5	21.75	6.74	0.12	1.06	2.595
Arithmetic mean	23.9	20.8	22.35	4.42	0.09	0.81	1.983
control	23.8	23.8	23.8	1.13	0.04	0.43	1.064
* Represents the average of three replicates.							

The results of Table (2) indicate that the nanomaterial prepared biologically using *Beauveria bassiana* fungus treated with silver nitrate solution had a significant effect on the amount of protein as the concentrations recorded 0.125, 0.250, 0.500 mg/L with a weight of 24.234, 127.84, 35.72 mg/g respectively, while the control treatment recorded 382.81 mg/g. While the above concentrations of the nanomaterial prepared using zinc oxide solution had an effect as they recorded 304.37, 233.125, 43.75 mg/g respectively, while the control treatment recorded 361.53 mg/g.

Table 2: Effect of different concentrations of the nanomaterial prepared biologically using the fungus Beauveria bassiana treated with silver nitrate and zinc oxide on the amount of protein in the body of the adult Periplaneta americana L.

Materials used / Concentration mg/l	proteins weight		Arithmetic mean	Variance	covariance coefficient	Standard error of the mean	Standard deviation
	silver nitrate	Zinc oxide					
0.125	*234.24	*304.37	269.30	1492	0.14	15.77	38.6
0.250	127.64	233.12	180.38	3344	0.32	23.61	57.8
0.500	35.72	43.75	39.73	25.32	0.13	2.05	5.03
Arithmetic mean	132.53	193.74	163.135	1620	0.19	13.47	33.81
control	382.81	361.53	372.17	148.39	0.03	4.97	12.18
* Represents the average of three replicates.							

The results of Table (3) indicate that the nanomaterial prepared biologically using *Beauveria bassiana* fungus treated with silver nitrate solution had a significant effect on the amount of protein as the concentrations recorded 0.125, 0.250, 0.500 mg/L with a weight of 234.24, 127.64, 35.72 respectively, while the control treatment recorded 382.81 mg/g. While the above concentrations of the nanomaterial prepared using zinc oxide solution had an effect as they recorded 304.37, 233.12, 43.75 mg/g respectively, while the control treatment recorded 361.53 mg/g.

Table 3: Effect of different concentrations of the nanomaterial prepared biologically using the fungus Beauveria bassiana treated with silver nitrate on the percentage of potassium in the body of adult Periplaneta americana

Materials used / Concentration mg/l	Percentage of potassium		Arithmetic mean	Variance	covariance coefficient	Standard error of the mean	Standard deviation
	silver nitrate	Zinc oxide					
0.125	* 4.65	* 5.14	4.89	0.62	0.16	0.32	0.78
0.250	2.36	3.21	3.28	0.65	0.28	0.33	0.80
0.500	0.54	0.75	0.64	0.06	0.38	0.10	0.21
Arithmetic mean	2.51	3.03	2.93	0.44	0.27	0.25	0.59

control	6.22	6.25	6.23	0.40	0.10	0.26	0.63
* Represents the average of three replicates.							

The results of Table (4) indicate that the nanomaterial prepared biologically using *Beauveria bassiana* fungus treated with silver nitrate solution had a significant effect on the amount of potassium element as the concentrations recorded 0.125, 0.250, 0.500 mg/L with a weight of 4.65, 2.36, 0.54 mg/g respectively while the control treatment recorded 6.22 mg/g. While the above concentrations of the nanomaterial prepared using zinc oxide solution had an effect as they recorded 5.14, 3.21, 0.75 mg/g respectively while the control treatment recorded 6.25 mg/g.

Table 4: Effect of different concentrations of the nanomaterial prepared biologically using the fungus Beauveria bassiana treated with silver nitrate and zinc oxide on the amount of sodium in the body of the adult Periplaneta americana.

Materials used / Concentration mg/l	Sodium percentage		Arithmetic mean	Variance	covariance coefficient	Standard error of the mean	Standard deviation
	silver nitrate	Zinc oxide					
0.125	* 2.63	* 2.45	2.54	0.32	0.23	0.23	0.56
0.250	1.22	1.08	1.15	0.38	0.53	0.25	0.61
0.500	0.46	0.42	0.44	0.10	0.63	0.13	0.31
Sodium percentage	1.43	1.31	1.37	0.26	0.46	0.20	0.49
control	3.24	3.31	3.27	0.44	0.20	0.27	0.66
* Represents the average of three replicates.							

The results of Table (4) indicate that the nanomaterial prepared biologically using *Beauveria bassiana* fungus treated with silver nitrate solution had a significant effect on the amount of sodium element as the concentrations recorded 0.125, 0.250, 0.500 mg/L with a weight of 2.63, 1.22, 0.46 mg/gm respectively while the control treatment recorded 3.24 mg/gm. While the above concentrations of the nanomaterial prepared using zinc oxide solution had an effect as they recorded 2.45, 1.08, 0.42 mg/gm respectively while the control treatment recorded 3.34 mg/gm.

The results of Table (5) indicate that the nanomaterial prepared biologically using *Beauveria bassiana* fungus treated with silver nitrate solution had a significant effect on the amount of phosphorus element as the concentrations recorded 0.125, 0.250, 0.500 mg/L with a weight of 4.32, 2.61, 0.40 respectively while the control treatment recorded 6.13 mg/g. While the above concentrations of the nanomaterial prepared using zinc oxide solution had an effect as they recorded 5.12, 3.43, 0.70 mg/g respectively while the control treatment recorded 6.24 mg/g.

Table 5: Effect of different concentrations of the nanomaterial prepared biologically using the fungus Beauveria bassiana treated with silver nitrate and zinc oxide on the amount of phosphorus in the body of the adult Periplaneta americana

Materials used / Concentration mg/l	phosphorus percentage		Arithmetic mean	Variance	covariance coefficient	Standard error of the mean	Standard deviation
	silver nitrate	Zinc oxide					

0.125	4.32	5.12	4.72	0.77	0.19	0.36	0.87
0.250	2.61	3.43	3.02	1.33	0.38	0.47	1.15
0.500	0.40	0.70	0.55	0.14	0.74	0.15	0.37
Arithmetic mean	2.44	3.07	2.75	0.74	0.43	0.32	0.76
control	6.13	6.24	6.18	0.42	0.11	0.27	0.65
* Represents the average of three replicates.							

4. Conclusion and future scope

Concentration

The results of this research demonstrated the ability Silver nanoparticles and Zinc nanoparticles prepared biologically by the fungus Beauveria bassiana on the feeding behavior and the insect body content of some materials and nutrients in the Periplaneta americana L.

Recommendations

Authors recommend the possibility of using nano-preparations such as silver and zinc nanoparticles bio-prepared by fungi as alternative materials to chemical pesticides in controlling insects.

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Reference

[1] Afridi, H.I.; Kazi, T.G.; Talpur, F.N. (2018).Correlation of Calcium and Magnesium Levels in the Biological Samples of Different Types of Acute Leukemia Children. Biol. Trace Element Res, 186, 395–406. [CrossRef] [PubMed].

[2] Ajai, A.I.; Bankole, M.; Jacob, J. O. and Audu, U. A. (2013). Determination of some essential minerals in selected edible insects. African Journal of Pure and Applied Chemistry, 7(5); 194-197.

[3] Ananthu .C.M . & Renjanadevi. B (2016) Preparation of Zinc OxideNanoparticles and its Characterization Using Scanning Electron Microscopy (SEM) . Procedia Technology 24 . 761 – 766.

[4] Daniel. K, Grace .M, Emmanuel .M, Amanda. S(2023). Fats and major fatty acids present in edible insects used as food and livestock feed. Veterinary and Animal Science 22(4).1-8.

[5] Goswami A, Roy I, Sengupta S, Debnath N (2010) Novel applications of solid and liquid formula-tions of nanoparticles against insect pests and pathogens. Thin Solid Films 519(3):1252–1257.

[6] Judith H Myers & Rana M. Sarfraz (2016). Effects of Insect Herbivores on Plant Populations , Annual Review of Entomology 62(1) DOI:10.1146/annurev-ento-010715-02382.

[7] Ksenija Nesic& Jutta Zagon.(2019). Insects a Promising Feed and Food Protein Source , Scientific Institute of Veterinary Medicine of Serbia, Meat Technology 60(1):56-67.

[8] Mansor, M. Sh, and N. M. Al-Mallah. "EFFECT OF SUB-LETHAL DOSE OF SOME INSECT GROWTH INHIBITORS ON SOME PHYSICAL FEATURES OF WINGS CUTICLE OF AMERCINCA COCKROACH PERIPLANETA AMERICANA." The Iraqi Journal of Agricultural Science 48.5 (2017): 1255-1262.

[9] Mona. h , Fatma el-zamek ,H. abd el-fattah, Samir M (2019) biosynthesis of zinc nanoparticles using culture filtrates

of aspergillus, fusarium and penicillium fungal species and their antibacterial properties against gram-positive and gram-negative bacteria Zagazig Journal of Agricultural Research 46(6):2009-2021.

[10] Papachova Z., Cahova M.(2015). Fatty acid signaling The new role of intracellular lipase. Int. J. Mol. Sci. 2015;16:3831–3855. doi: 10.3390/ijms16023831. [PMC free article] [PubMed] [CrossRef] [Google Scholar].

[11] Renske H. Janssen, Jean-Paul Vincken, Lambertus A.M. Van Den Broek (2017). Nitrogen-to-Protein Conversion Factors for Three Edible Insects: Tenebrio molitor, Alphitobius diaperinus, and Hermetia illucens .Journal of Agricultural and Food Chemistry 65(11). 2275-2278.

[12] Saadbin Khan & Khalid Hossain. (2022). Classification and Properties of Nanoparticles. Nanoparticle-Based Polymer Composites1stChapter :pp.15-54.

[13] Sara A. Gezaf, Hend A. Hamedo, Amira A. Ibrahim, Monga I. Mossa(2022). Mycosynthesis of silver nanoparticles by endophytic fungi: Mechanism Microbial Biosystems 7(2) 185718.1066.

[14] Sargenti, A.; Farrugia, G.; Zaccheroni, N.; Marraccini, C.; Sgarzi, M.; Cappadone, C.; Malucelli, E.; Procopio, A.; Prodi, L Lombardo, M.; et al. Synthesis of a highly Mg2+-selective fluorescent.; probe and its application to quantify and imaging total intracellular magnesium. Nat. Protoc. 2017, 12, 461–471.

[15] Subhan Allah Khan and Minglin Lang.(2023) A comprehensive review of the roles of metals mediating interactions between insects and microbial pathogens, Wen Liu, Academic Editor July 23; 13(7): 839-848.

[16] Ursula. S, Niclas T, Florian. N,& Heinz. R(2019). ATP as Phosphorus and Nitrogen Source for Nutrient Uptake by Fagus sylvatica and Populus x canescens RootsData, Frontiers in Plant Science , Volume 10 Article 378 pp 1-15.

[17] Victor.B. Meyer.R, Ruparao. T, Gahukar. S. G, and Chuleui. J (2021) Chemical Composition, Nutrient Quality and Acceptability of Edible Insects Are Affected by Species, Developmental Stage, Gender, Diet, and Processing Method Published online 2021 May 10. doi: 10.3390/foods10051036.

[18] Zai-Ling Wang, Xiang-Ping Wang, Chuan-Ren Li, Zhen-Zhou Xia, and Shi-Xiang Li.(2018) Effect of Dietary Protein and Carbohydrates on Survival and Growth in Larvae of the Henosepilachna vigintioctopunctata (F.) (Coleoptera: Coccinellidae) J Insect Sci. 18(4): 3.1-18..