CRESPO, R., JAUREZ, M.P., DAL BELLO, G.M., PADIN, S., CALDERON FERNANDEZ, G., PEDRINI, N., 2002. Increased mortality of Acanthoscelides obtectus by alkane-grown *Beauveria bassiana*. BioControl 47, 685–696.

ER, M.K., H. TUNAZ, A.A. IŞIKBER, 2016. Improving the virulence of a native *Beauveria bassiana* isolate against *Rhyzopertha dominica* adults. 7th International Scientific Agriculture Symposium (Agrosym 2016). 6-9 October 2016, Jahorina, Bosnia and Herzegovina. pp: 1464–1469 DOI:10.7251/AGRENG1607221.

FERIZLI, A. G., BERIS, G., BASPINAR, E., 2005. Mortality and F1 production of *Rhyzopertha dominica* (F.) on wheat treated with diatomaceous earth; impact of biological and environmental parameters on efficacy. Journal of Pest Science 78, 231–238.

HAQ, T., USMANI, N. F., ABBAS, T., 2005. Screeening of plant leaves as grain protectants against *Tribolium castaneum* during storage. Journal of Botany **37**, 149–153.

KHASHAVEH, A. and H. CHELAV, S., 2013. Laboratory bioassay of Iranian isolates of entomopathogenic fungus *Metarhizium anisopliae* (Metsch.) Sorokin (Ascomycota: Hypocreales) against two species of storage pest. Agriculturae Conspectus Scientificus **78**, 35–40.

MICHALAKI, M. P., ATHANASSIOU, C. G., TEENBERG, T., BUCHELOS, C.Th., 2007. Effect of *Paecilomyces fumosoroseus* (Wise) Brown and Smith (Ascomycota: Hypocreales) alone or in combination with diatomaceous earth against *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae) and *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae). Biological Control **40**, 280–286.

MOINO, JR A., ALVES, S.B., PEREIRA, R.M., 1998. Efficacy of *Beauveria bassiana* (Balsamo) Vuillemin isolates for control of stored-grain pests. Journal of Applied Entomology **122**, 301–305.

MOORE, D., LORD, J. C., SMITH, S. M., 2000. "Pathogens, 193-227". In: Alternatives to Pesticides in Stored-Product IPM (Eds: B. H. Subramanyam & D. W. Hagstrum). Kluwer Academic Publishers, Dordrecht Netherlands, 437 pp.

PADIN, S., BELLO, G. D., FABRIZIO, M., 2002. Grain loss caused by *Tribolium castaneum*, *Sitophilus oryzae* and *Acanthoscelides obtectus* in stored durum wheat and beans treated with *Beauveria bassiana*. Journal of Stored Products Research **38**, 69–74.

PEDRINI, N., DAL BELLO, G.M., PADIN, S.B., JAUREZ, M.P., 2011. Insecticidal capacity of hydrocarbon-grown *Beauveria bassiana* to control coleoptera in stored grain. Agrociencia Uruguay **15**, 64–69.

RIASAT, T., WAKIL, W., ASHFAQ, M., SAHI, S. T., 2011. Effect of *Beauveria bassiana* mixed with diatomaceous earth on mortality, mycosis and sporulation of *Rhyzopertha dominica* on stored wheat. Phytoparasitica 39, 325–331.

RIASAT, T., WAKIL, W., YASIN, M., KWON, Y. J., 2013. Mixing of *Isaria fumosorosea* with enhanced diatomaceous earth and bitterbarkomycin for control of *Rhyzopertha dominica*. Entomological Research 43, 215–223.

SEWIFY, G.H., EL SHABRAWY, H.A., EWEIS, M.E., NAROZ, M.H., 2014. Efficacy of entomopathogenic fungi, *Beauveria bassiana* and *Metarhizium anisopliae* for controlling certain stored product insects. Egyptian Journal of Biological Pest Control 24, 191–196.

SHAFIGHI, Y., ZIAEE, M., GHOSTA, Y., 2014. Diatomaceous earth used against insect pests, applied alone or in combination with *Metarhizium anisopliae* and *Beauveria bassiana*. Journal of Plant Protection Research 54, 62–66.

SHAMS, G., SAFARALIZADEH, M. H., IMANI, S., SHOJAI, M., ARAMIDEH, S., 2011. A laboratory assessment of the potential of the entomopathogenic fungi *Beauveria bassiana* (Beauvarin) to control *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) and *Sitophilus granarius* (L.) (Coleoptera: Curculionidae). African Journal of Microbiology Research 5, 1192–1196.

STEJSKAL, V., HUBERT, J., AULICKY, R., KUCEROVA, Z., 2015. Overview of present and past and pest-associated risks in stored food and feed products: European perspective. Journal of Stored Products Research 64, 122–132.

WAKIL, W. and GHAZANFAR, M. U., 2010. Entomopathogenic fungus as a biological control agent against *Rhyzopertha dominica* F. (Coleoptera: Bostrychidae) on stored wheat. Archives of Phytopathology and Plant Protection 43, 1236–1242.

WAKIL, W. and SCHMITT, T., 2014. Field trials on the efficacy of *Beauveria bassiana*, diatomaceous earth and Imidacloprid for the protection of wheat grains from four major stored grain insect pests. Journal of Stored Products Research 64, 160–167.

WAKIL, W., RIASAT, T., GHAZANFAR, M. U., KWON, Y. J., SHAHEEN, F. A., 2011. Aptness of *Beauveria bassiana* and enhanced diatomaceous earth (DEBBM) for control of *Rhyzopertha dominica* F. Entomological Research 41, 233–241.

Bio-nanosilver synthesized by the entomopathogenic nematode-symbiotic bacterium as bio-insecticide for the red flour beetle (*Tribolium castaneum)*

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Abstract

Biological control can be another important way to manage post-harvest insect pests. Some organisms that showed biological control activity against some soil pests are insect-parasitic nematodes. There are two different species of nematodes, steinernematids and heterorhabditids, who carry within their bodies insect-pathogenic bacteria. *Xenorhabdus* spp are bacteria which infest steinernematids and *Photorhabdus* spp. bacteria infect heterorhabditids. The study aimed to develop pesticide alternatives by synthesizing silver bio-nanoparticles (AgNPs) using *Xenorhabdus indica* bacterial filtrate. The nanoparticles synthesized by the bacterial strains were purified and its cytotoxicity and bioactivity was examined against the larvae of the *Tribolium castaneum*. AgNPs were characterized by Scanning Electron Microscopy and X-Ray diffraction analysis, and the results revealed that the obtained nanoparticles are nanosilver with sizes ranging from 30 to 70 nm, with spherical shape and non-smoothed surface. Insect larvae were initially exposed to descending concentrations (100, 50, 25, 10 and 5 µg/ml) of the biosynthesized nanosilver for 48 hours. Results of the bioassay showed that mortality of treated larvae was concentration-dependent with LC_{50} of 25 µg/ml. Higher mortality percentage (89%) was observed with the concentration 100 ug/ml and the lower one was obtained by the concentration 5 ug/ml (60%). Subsequently, data of the present study suggest these bio-AgNPs-bacterial filtrate complexes could be used as potentially effective eco-friend bio-control candidates. However, testing other types of bio-synthesized nanomaterials, and its vital effect as bio-insecticide for storage insect species are still under investigation.

Keywords: Entomopathogenic bacteria, biocontrol, nanosilver, *Tribolium* sp., *Xenorhabdus* sp.

Introduction

Stored commodities are vulnerable towards attack of insects (Ukeh *et al*., 2012) and a possible infestation can deteriorate the quality as well as the quantity of the attacked commodity (Nadeem *et al.,* 2012). This will resulted in significant decrease in volume, nutritional value, substantial weight loss and reasonable germination damage (Nadeemet al., 2012, Phillips et al., 2010). It was reported that *Tribolium castaneum* is a common pest found in granaries, mills, warehouses, especially in wheat flour, which causes serious damages to all kinds of stored grain products (Prakash *et al.,* 2008), but also feeding on different stored-grain and grain products (Weston and Rattlingourd, 2000). Being polyphagous and cosmopolitan, a number of insecticides had been used for successful control of this pest (Islam and Talukdar, 2005).

Synthetic insecticides have been successfully used to protect stored grains from insect infestation (Sighamony. *et al*., 1986). *T. castaneum* is affected by both the quantity and quality of synthetic insecticides such as malathion, pirimiphos-methyl, chlorpyrifos-methyl, deltamethrin and the fumigant phosphine. These are currently the main products used to protect stored grains from insects (Bond, 1984). Increased public concern over the residual toxicity of insecticides applied to stored products, the occurrence of insecticide-resistant insect strains, and the precautions necessary to work with traditional chemical insecticides stress the usage of e.g. botanicals to control insects of stored product (Su, 1991).

In the present decade, nanotechnology is a promising field that introduces an excellent chance for research and is expected to give major impulses to technical innovations in a variety of industrial sectors in the future. Benelli (2016) reported that the biosynthesis of AgNPs is an arising tool for fighting mosquito vectors. Nanoparticles of noble metals like silver and gold exhibited remarkable physical, chemical and biological properties from their bulk counter parts (Priya and Santhi, 2014).

Microbial and endo-toxin insecticides based on *Bacillus* spp. bacteria, as well as decreasing of breeding habitat can achieve considerable IPM program goals (Rydzanicz *et al*., 2009). In this context, Adams and Nguyen (2002), found that *Xenorhabdus* and *Photorhabdus* gram negative symbiotic bacteria accompanied with the entomopathogenic nematodes *Steinernema* and *Heterorhabditis*, are injected into the haemocoel of target insect hosts. A variety of these toxins have been characterized and classified into four major groups (Rodou *et al*., 2010). The toxin complexes (Tcs) are one of these four major groups which attracted attention from the fact that some of their complexes showed a high potential toxicity towards insects after oral application, suggesting potentiality as insecticides (Waterfield *et al*., 2001).

Results and Discussion

The results revealed that the obtained nanoparticles are nanosilver with sizes ranging from 30 to 70 nm, with spherical shape and non-smoothed surface. Insect larvae were initially exposed to descending concentrations (100, 50, 25, 10 and 5 µg/ml) of the biosynthesized nanosilver for 48 hours. Results of the bioassay showed that mortality of treated larvae was concentration-dependent with LC_{50} of 25 µg/ml. Higher mortality percentage (89%) was observed with the concentration 100 ug/ml and the lower one was obtained by the concentration 5 ug/ml (60%). Subsequently, data of the present study suggest these bio-AgNPs-bacterial filtrate complexes could be used as potentially effective eco-friend bio-control candidates.

The cause of insect death could be *via* binding the nanoparticles to proteins containing sulphur in the intracellular space or phosphorus in the DNA, which leads to enzyme and organelle degradation. Basically, cell death is mainly caused by decreased membrane permeability and disturbed proton motive force which leads to cellular function loss. The pathogenicity of these toxin complexes upon releasing into the host heamolymph causes histopathological lesions and septicaemia leading to host death. Moreover, the high larvicidal activity of AgNPs can be attributed to their lower particle size which increases the surface area to volume ratio, and thus, increases its action against insect.

Conclusion

Subsequently, data of the present study suggest these bio-AgNPs-bacterial filtrate complexes could be used as potentially effective eco-friend bio-control candidates. However, testing other types of bio-synthesized nanomaterials, and its vital effect as bio-insecticide for storage insect species still under investigation.

Tab 1: The effect of the culture filtrate and the biosynthesized nanosilver on insect mortality compared with a commercial insecticide.

		Mean mortality± SE					
	*Concen-tration **(%)***	*Time **(hrs)***					
		3hr	***6hr***	***12hr***	***24hr***	***48hr***	***72hr***
Water	*Cont.*	*0.00±0.00*	*0.00±0.00*	*0.00±0.00*	*0.00±0.00*	*0.00±0.00*	*0.00±0.00*
Culture filtrate	*1*	*0.33±0.577*	*15.0±10.0*	*23.33±12.58*	*30.0±10.0*	*35.0±15.0*	*50.0±10.0*
	10	*0.00±0.00*	*26.67±2.9*	*31.667±2.89*	*41.67±2.89*	*51.667±2.89*	*65.0±5.0*
	20	*66.67±18.9*	*75.0±22.9*	*78.33±20.82*	*81.667±15.*	*83.33±12.58*	*90.0±10.0*
Insecticide	*1*	*0.0±0.0*	*1.67±2.89*	*5.0±0.0*	*13.3±2.887*	*16.667±2.89*	*26.67±2.9*
	10	*0.0±0.0*	*3.3±2.887*	*13.33±7.638*	*23.33±2.887*	*30.0±0.0*	*50.0±5.0*
	20	*56.667±16.*	*66.67±16.*	*75.0±17.32*	*78.3±15.28*	*80.0±18.028*	*86.7±12.5*
	1	*0.00±0.00*	*0.00±0.00*	*0.00±0.00*	*6.67±2.887*	*11.667±2.89*	*21.67±2.9*
Bio-synthesized nano silver	*10*	*11.667±7.6*	*23.33±10.*	*35.0±13.229*	*53.33±10.4*	*60.0±13.229*	*73.33±10.*
	20	*90.0±5.0*	*90.0±5.0*	*93.33±2.887*	*95.0±5.0*	*98.33±2.887*	*100.0±0.0*

References

ADAMS, B.J. and K.B. NGUYEN, 2002: Taxonomy and Systematics. Pp. 1–34 in R. GAUGLER (ed.) Entomopathogenic Nematology. CAB International, Wallingford, UK.

BOND, E., 1984: Manual of fumigation for insect control. – Food and Agriculture Organization of the United Nation, Rome, 432 pp.

FRENCH-CONSTANT, R.H., DOWLING, A. and WATERFIELD, N.R., 2007. Insecticidal toxins from *Photorhabdus bacteria* and their potential use in agriculture. Toxicon **49**: 436–451.

ISLAM, M.S. and F.A. TALUKADER 2005: Toxic and residual effects of *Azadirachta indica*, *Tagetes erecta* and *Cynadon dactylon* seed extract and leaf powders towards *Tribolium castaneum*. Journal of plant diseases and protection **112**(6).

NADEEM, M., IQBAL, J., KHATTAK, M.K. and M.A. SHAHZAD, 2012. Management of *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) using neem *Azadirachta indica* (A. Juss) and tumha *Citrullus colocynthis* (L.). Pakistan Journal of Zoology **44**: 1331–325.

PHILIPS, T.W. and J.E. THRONE, 2010. Bio-rational- Approaches to Managing Stored Product. Annual Review of Entomology **55**: 375–397.

PRAKASH, A., RAO, J. and V. NANDAGOPAL, 2008. Future of Botanical Pesticides in rice, wheat, pulses and vegetables pest management. Journal of Biopesticides **1**(2): 154–169.

RODOU, A., ANKARAH, D.O., and C. STATHOPOULOS, 2010. Toxins and secretion systems of *Photorhabdus luminescens*.Toxins **2**: 1250–1264.

RYDZANICZ, K., LONC, E. and N. BECKER, 2009. Current procedures of the integrated urban vector mosquito control as an example in Cotonou (Benin, West Africa) and Wroclaw area (Poland). Wiad Parazytol **55**(4): 335–340.

SIGMANONY, S., ANEES, I., CHANDRAKALA T.S. and Z. OSMANI, 1986. Natural products as repellents for *Tribolium castaneum* (Herbst). International Pest Control **26**: 156–157.

UKEH, D.A., OKU, E.E., UDO, I.A., NTA, A.I. and UKEH, J.A., **.** 2012. Insecticidal effect of fruit extracts from *Xylopia aethiopica* and *Dennettia tripetala* (Annonaceae) against *Sitophilus oryzae* (Coleoptera: Curculionidae). Chilean Journal of Agricultural Research **72**: 195–200.

WATERFIELD, N.R., BOWEN, D.J., FETHERSTON, J.D., PERRY, R.D., and R.H. FRENCH-CONSTANT, 2001. The tc genes of *Photorhabdus*: a growing family. Trends Microbiology **9**: 185–191.

WEBSTER, J.M., CHEN, G. and L.J. HUK, 2002: Bacterial metabolites.Entomopathogenic nematology. Pp. 99–114 in R. GAUGLER (ed.) Entomopathogenic Nematology. CAB International, Wallingford, UK.

WETSON, P.A. and P.A. RATTLINGOURD, 2000. Progeny production by *Tribolium castaneum* (Coleoptera: Tenebrionidae) and *Oryzaephilus surinamensis* (Coleoptera: Silvanidae) on maize previously infested by *Sitotroga cerealella* (Lepidoptera: Gelechiidae). Journal of Economic Entomology **93**: 535–533.

WHITE, N.D.G. 1995: Insects, mites, and insecticides in stored grain ecosystems. – in: JAYAS, D.S., WHITE, N.D.G. and W.E. MUIR (Eds.), Stored Grain Ecosystem. Marcel Dekker, Inc., New York 123–168.

Insecticidal Effect of Central Anatolian Region Diatomaceous Earths Against Confused Flour Beetle (*Tribolium confusum* Du Val.) on Stored Paddy

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Abstract

In this study, insecticidal efficacy of different local diatomaceous earth (DE) deposits obtained Central Anatolian Region in Turkey and commercial DE deposit (German origin), Silicosec® were evaluated against substantial pest on stored grain as *Tribolium confusum* du Val (Coleoptera: Tenebrionidae) at five different concentrations of 100, 300, 500, 900 and 1500 ppm on stored paddy. Mortality of the exposed adults was assessed after 7, 14 and 21 days of exposure. Also progeny productions were assessed after 65 days The tests were carried out at 25±1 oC temperature, 55±5% R.H. under dark conditions. The most effective DE in a short time were assessed AG2N-1 which caused 97% mortality of *T. confusum* adults at 1500 ppm concentration after 7 days of exposure in paddy. Complete mortality of *T. confusum* adults was recorded on AG2N-1 at 900 ppm for 14 days and treatments of AG2N-1, BGN-1, CBN-1 for 21 days at 500, 900 and 1500 ppm respectively whereas 87% mortality rate was determined for 21 days exposure of Silicosec® at the highest concentrations on paddy. In conclusion, this study indicated that Turkish DE deposits, AG2N-1, BGN-1 and CBN-1 had high insecticidal efficacy in comparison with the commercial Silicosec® and would have potential to be used against insects in the pest management of stored paddy.

Keywords: Turkish diatomaceous earths, *Tribolium confusum*, toxicity, paddy, Silicosec

1. Introduction

Currently, the control of insect pests in durable stored food products, such as grains and legumes, is based on the use of chemical methods such as fumigants and residual insecticides. However, the use of these substances is directly related with toxic residues on the final product, as well as serious environmental hazards. These factors, along with the consumers' demand for residue-free food and the development of resistance by several insect pests, have made essential the evaluation of alternative, low-risk and environmentally-friendly control methods. One of the most promising alternatives over the use of traditional pesticides in durable stored products is the use of diatomaceous earths (DEs). DEs are composed by the fossil skeletons of phytoplanktons, also known as diatoms, which occur in fresh and salt water since the Eocene period and produce a soft sedimentary rock, which is composed mainly by amorphous silica (SiO_2 + H_2O). The DEs currently mined vary remarkably in their insecticidal activity, depending upon species composition, geological and geographical origin as well as certain chemical characteristics, such as SiO2 content, pH and tapped density (Korunic 1997). DEs are probably the most efficacious natural resource-based dry materials that can be used as insecticides (Korunic 1998). DEs act in the insects' exoskeleton (cuticle) causing rapid desiccation resulting in death through water loss. They are non-toxic to mammals (rat oral LD50>5000 mg/kg of body weight), leave no toxic residues on the product and