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Effect of entomopathogenic fungi, *Hirsutella thompsonii* on mortality and detoxification enzyme activity in *Periplaneta Americana*

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Abstract
Insecticides have been broadly used to control cockroaches but these insects have developed resistance to several pesticides. Entomopathogenic fungi can be ideal for the biocontrol of cockroaches as it is environment-friendly microbial pesticide. The aim of the present study is to determine the effect of *Hirsutella thompsonii*, an entomopathogenic fungus on mortality and antioxidant enzymes of *Periplaneta Americana*. Conidial suspensions of fungi were given to cockroaches through different modes for 24 hours. We observed the positive effect of *H. thompsonii* on both mortality and the antioxidant enzyme (catalase, superoxide dismutase and glutathione peroxidase) activity in different tissues of cockroaches. The mortality rate in cockroaches ranged between 54.98% and 63.45% when treated with *H. thompsonoii* through different modes. The results showed that the enzyme activity of catalase (1.23±0.1 to 17±0.3 U/mg pot.), superoxide dismutase (3.3±0.3 to 20.1±0.3 U/mg prot.) and glutathione peroxidase (2.2±0.1 to 11.7±0.2 U/mg prot.) varied in different tissues of cockroaches. The present study reveals that the *H. thompsonii* have great potential for biocontrol against insects.

Keywords: *Hirsutella thompsonii*, *Periplaneta Americana*, Mortality, Antioxidant enzymes, Biocontrol.

Introduction
Periplaneta Americana (Linnaeus) (Blattodea: Blattidae) are considered as vectors of several human diseases and prefer warm temperatures around 29°C for survival. Cockroaches are medically related with many illnesses and health problems [1]. They carry viral and bacterial pathogens on their bodies and faeces which can cause poisoning, diarrhea and dysentery [2]. Cockroaches feed on garbage and sewage and have abundant opportunities to spread human pathogens [3, 4].

Chemical pesticides have been used constantly against pests which show resistance to the pesticide action [5]. The severe use of these chemicals has harmful effects on beneficial insects, human beings and other animals. Such problems have become a rationale for searching of safe pesticides [6]. The biological control of insects, especially use of entomopathogenic fungi is a very potential alternative to ensure efficient pest control. More than 700 species of fungi are entomopathogenic agents against insects [7]. Entomopathogenic fungi act by contact of fungal conidia with the host cuticle and penetrates through the integument into the haemocoel where it encounters with the potential cellular and humoral immune responses of the host. Proliferation of the fungal spores within haemocoel depends on the potential of the entomopathogenic fungi to overcome or avoid host's immune responses [8]. *Hirsutella thompsonii* [9], a mononematous species has hosts globally and during hot, humid weather can cause spectacular natural epizootics amongst host and is considered to be a major natural enemy of various pests [10].

Reactive oxygen species (ROS) are produced in all aerobic cellular metabolic processes and is potentially harmful to cells, causing oxidation of lipids, proteins and DNA as a result of the obligatory dependence of oxidative metabolism on molecular oxygen [11]. ROS includes the hydrogen peroxide (H_2O_2), hydroxyl radical ($^{\bullet}OH$), and the superoxide radical ($^{\bullet}O2^{-}$), all of which affect mainly lipids, proteins, carbohydrates, and nucleic acids [12]. The Insects possess an antioxidant enzyme protection which is strategically similar to that of vertebrates [13]. The antioxidant system comprises of numerous enzymes such as catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx). The SOD converts superoxide radical into H_2O_2 and molecular oxygen and the accumulation of H_2O_2 is prevented in the cell by CAT and GPx. The action of mechanism of the activities of SOD, CAT, and GPx are confirmed

both in plant species [14, 15] and in animal species [16, 17] but less is known about the activity of antioxidant enzymes in insects, especially *P. americana* in response to stress of entomopathogenic fungi, *H. thompsonii.* It attacks a number of tetranychid and eriophid mites of many crop plants in nature [18, 19]. The objective of the present study was to evaluate the efficacy of the *H. thompsonii* on mortality and the role of oxidative biomarkers in the *P. Americana.*

2. Materials and methods

2.1Insects rearing

The target insect *P. Americana* used in the present study originated from the different natural habitats like godowns, stores and streets. The adult cockroaches were kept in wooden boxes at temperature of 20 to 25 ºC and 35 to 50% of humidity for 24 hours (14 hours in light and 10 hours in dark). Insects were fed by biscuits and a piece of cotton soaked with water was used as a water source. Food and water sources were changed after 2 days and wooden boxes were cleaned after 10 days. Insects were sacrificed after 24 hours after exposure with spores.

2.2 Culture of entomopathogenic fungi

Entomopathogenic fungi, *H. thompsonii* (M.T.C.CNo.3556) were obtained from the culture collection of M.T.C.C. Chandigarh, India. Stock cultures of the isolates were stored at -80ºC. *H. thompsonii* was cultured on potato carrot agar media in petri dishes and after inoculation the fungi in media plates were placed in BOD incubator at 28±1 ºC for 14 days to complete the sporulation.

2.3 Preparation of fungal solution

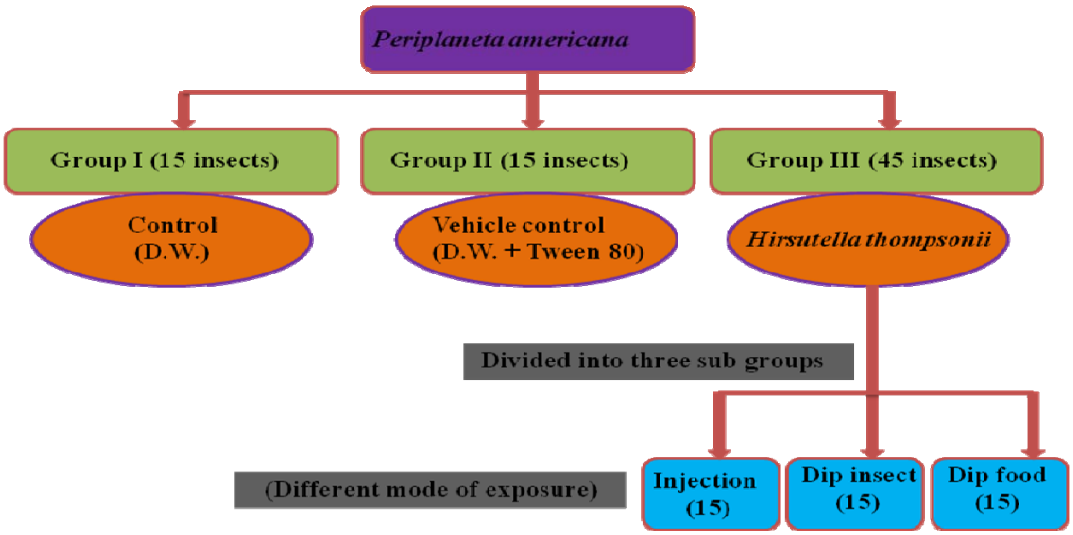
A mixture of conidia and hyphae were harvested by flooding the Petri dishes with sterile distilled water containing 0.05% (v/v) Tween 80 and kept the fungal solution in rotatory shaker at 26 ºC at room temperature for 1/2 hour in 150 × g. Fungal solution were kept overnight at 4 ºC before use. Conidial were separated from hyphae and substrate materials by filtration of the suspension through a five layer of cheese cloth. Conidial concentration was counted with haemocytometer (improved Neubauer, 0.1mm depth) and spore concentration of 4.2×10^9 spores per millilitre was prepared from stock solution.

2.4 Mode of treatments of insects with fungal spore

The experimental insects were divided into three groups. Insects of group Ist and 2nd were treated with distilled water and Tween 80 known as control and vehicle control having 15 insects in each group respectively. Group third were divided into three sub-groups: injection mode, dip insect and dip food having 15 insects each.

Spore concentration of 4.2×10^9 spores per millilitre was prepared and insects were treated in three different ways, by direct contact with spore suspension mass through injection mode, through inter segmental region of 5th and 6th abdominal segment (15insects), food dipped with fungi spores (15 insects) and the insects were dipped (15 insects) in spore suspension of fungi.

2.5 Experimental design



2.6 Fungal susceptibility tests

Mortality in treatments and controls were recorded daily until end of the experiment. The dead insects were transferred to humid Petri dishes lined with moistened filter paper and incubated for five days for observation. Only those insects covered with mycelia and spores were considered to have died as a result of fungal infection. Percent mortality was calculated according to Abbott's formula [20].

$$\text{Abbott's corrected mortality} = \frac{\text{\% mortality in treatment - \% mortality in control}}{\text{100 - \% mortality in control}} \times 100$$

2.7 Insect dissection and tissue preparation for biochemical estimation.

After treated with the 4.2×10^9 spores per milli litre for 24 hours, *P. americana* were dissected to collect midgut and fatbody. The fat body and mid gut were washed in saline and homogenized in homogenizer 1M potassium phosphate buffer, pH 7.4 (according to weight). Haemolymph was collected from an antenna and prolegs into eppendorf tubes precooled to 4 ºC with several crystals of phenylthiourea and anticoagulant buffer (93 mM NaCl, 0.1M glucose, 30mM tri sodium citrate, 26 mM citric acid and 10 mM EDTA at pH 4.6) to prevent melanisation. All homogenates were centrifuged for 30 minutes at 4 ºC at 15000 × g and haemolymph was centrifuged for 10 min at 4 ºC at 500 × g, respectively. The supernatant was used for subsequent analysis.

2.6.1 Determination of protein concentration

Protein concentration of midgut, fatbody and haemolymph were determined by Lowry *et al* [21]. BSA was used to construct the calibration curve.

2.6.2 Assay of the antioxidant enzymes

Catalase activity was measured following an earlier method [22] with some modifications. Briefly, reaction mixture 1ml consisted of 0.05 M phosphate buffer (pH 7.0) and 0.003% H_2O_2. By adding diluted cell extract, reaction was started and decrease in absorbance at 240 nm was recorded for 10 min. Unit of the enzyme was defined as µmol of H_2O_2 depleted/min. The activity was expressed as units/mg protein.

The activity of superoxide dismutase (SOD) was measured by the method of Beauchamp *et al*., [23] with minor modification. Briefly, the reaction mixture contained 13 µM methionine, 13 µM riboflavin, 75 µM NBT and 50 mM phosphate buffer (pH 7.8), and 0.02 ml of enzyme extract. Instead of NBT, the mixture contained a phosphate buffer to serve as control. The absorbance was read at 560 nm. The volume of enzyme extract corresponding to 50% inhibition of the reaction was considered to be one enzyme unit.

Glutathione peroxidase (GPx) activity was determined as described earlier [24] with slight modifications. Briefly, cell extracts were added to the assay buffer consisting of 100 Mm Tris-Cl (pH 7.2), 3 mM EDTA, 1 mM sodium azide, 0.25 mM H_2O_2, 0.5 mM NADPH, 0.17 mM GSH, and 1 unit GR. The change in absorbance per minute at 340 nm was recorded for 10 min and enzyme activity was expressed as µmole NADPH oxidized/min/ mg protein.

3. Statistical Analysis

Experimental data were expressed as mean±SD and Student's t-test was applied for determining the level of significance between vehicle control vs. treated groups Fisher [25].

4. Results

4.1. Virulence of *H. thompsonii* for *P. americana*

In the present study, *H. thompsonii* were tested against cockroaches to study mortality and oxidative stress of the insect. Mortality was checked daily and dead insects were removed and placed on suitable medium at 28 ±1 ºC for an additional 2 days to check for signs of mycosis.

Table 1: Cumulative mortality percentage (%) of *P. americana* treated by *H. thompsonii* ***P<0.001 (vehicle control vs. treated groups, C-control, VC-vehicle control, I-injection, DI- dip insect, DF-dip food).

Cumulative Mortality (%)				
D.W.	**Tween 80**	***H. thompsonaii***		
C	**VC**	**I**	**DI**	**DF**
1.1±0.34	1.32±0.23	63.45±1.89***	57.87±1.22***	54.98±1.33***

The mortality within the control group was very low (1.1-1.32%) and no fungal growth were observed on dead control insects. Percentage mortality of insects was dependent on mode of exposure and it increases while applying by injection route, followed by spraying on cuticle (Table 1)

4.2. Effect of *H. thompsonii* on catalase activity in midgut, fat body and haemolymph of *P. Americana.*

Catalase activity decreased significantly (p<0.001) in midgut and hemolymph when exposed to fungal conidial, whereas, it's activity increased significantly (p<0.01) almost 2-3 folds in fat body when exposed to *H. thompsonii* (Figure 1a).

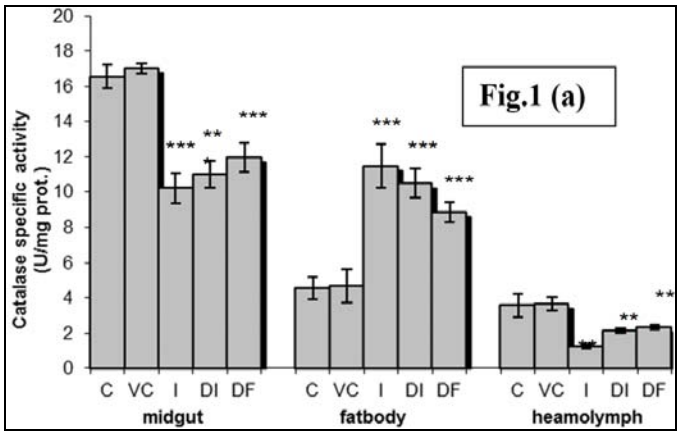


Fig 1: (a) Effect of *H. thompsonii* on specific activity of catalase on midgut, fatbody and haemolymph of *P. americana* (mean ± SD, n=4). ** P < 0.01, ***P<0.001 (vehicle control vs. treated groups). C-control, VC-vehicle control, I-injection, DI- dip insect, DF-dip food.

4.3. Effect of *H. thompsonii* on SOD activity in midgut, fatbody, and haemolymph of *P. americana.*

SOD activity decreased significantly (p<0.001) almost 2-3 times in midgut, fatbody and haemolymph when exposed *H. thompsonii* through injection route, whereas, SOD activity increased significantly (p<0.01) in all insects when exposed to the fungi through other routes (Figure 1b).

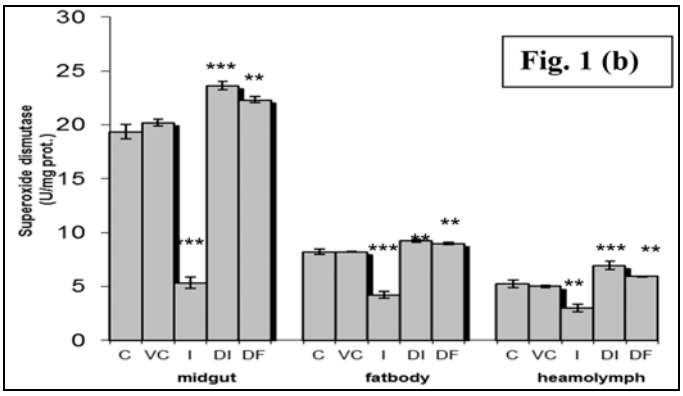


Fig 1(b): Effect of *H. thompsonii* on specific activity of superoxide dismutase on midgut, fatbody and haemolymph of *P. americana* (mean ± SD, n=4). ** P < 0.01, ***P<0.001 (vehicle control vs. treated groups). C- control, VC-vehicle control, I-injection, DI- dip insect, DF-dip food

4.4. Effect of *H. thompsonii* on GPx activity in midgut, fatbody, and haemolymph of *P. americana.*

The GPx activity showed significantly (p<0.001) an increased trend about 2-3 fold in all the three tissues (midgut, fatbody and heamolymph) when exposed to *H. thompsonii* (Figure 1c).

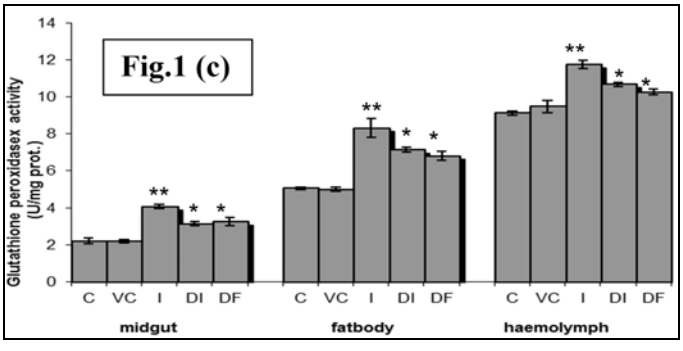


Fig 1(c): Effect of *H. thompsonii* on specific activity of glutathione peroxidase on midgut, fatbody and haemolymph of *P. americana* (mean ± SD, n=4). ** P < 0.01, ***P<0.001 (vehicle control vs. treated groups). C- control, VC-vehicle control, I-injection, DI- dip insect, DF-dip food.

5. Discussion

Conidial growth and post-mortem mycelial revealed that the fungal pathogens were the reason of insect's death. Higher mortality through injection mode might be due to the penetration of fungal spores into the host body without facing the first line of immune defense system from cuticle. Our result is in agreement with Poprawski [26] which showed that conidia of an *M. anisopliae* strain (ARSEF 759) causes mortality in brown citrus aphid. Mortality of cockroaches might be that, the fungal conidia along with the toxic action of various metabolites secreted by the fungus during the course of infection in the cockroach.

Reactive oxygen species induces oxidative stress by inducing cellular damage, which are acted upon by the enzymes of the antioxidant system, such as CAT, SOD and GPx [27, 28]. An alteration in the activity of SOD, CAT, and GPx were observed in *P. americana* after *H. thompsonii* treatment (Figures 1a,b,c) which suggest that *H. thompsonii* causes oxidative damage in cockroaches, probably by generating reactive-oxygen stress in their bodies, similar findings for SOD, CAT, and GPx has also been observed by (Fornazier *et al*. [29]; Lee and Shin [14].

In the present study an increased level of CAT activity has been observed in the midgut of *P. americana* which might be an effect of the high resistance of CAT to overcome the generation of free radicals. Naren Babu *et al*., [30] had also recorded the peak activity of CAT at 4th h in *P. American a*fter treated with fungal conidia of *Metarhizium anisopliae.* Similar results have been observed by Adamski,*et al*. [31] for CAT activity in the LC_{10} group on day 4 of *S. exigua* exposed to fenitrothion**.** In the present study catalase activity has declined in fatbody and hemolymph as compared to control which may be one of the reasons that causes the high mortality rate of cockroaches treated with *H. thompsonii.* Decrease in the CAT activity in fatbody and haemolymph through different routes implies unsuccessful role of the enzyme in controlling the oxidative stress induced by the fungus.Our findings are similar to earlier reports, wherein, catalase enzyme activity was declined in the hemolymph of silkworm after 24 hours of the inoculation of fungal pathogen *Beauveria bassiana* [32]. Allen, *et al*., [33] has also reported the inhibition of CAT by treatment of *Musca domestica* with 3-amino-1,2,4-triazole**.**

SOD activity has increased in midgut, fatbody and hemolymph of the fungal doses applied through dip insect and dip food, suggests that SOD was stimulated by scavenging $^{\bullet}O2^{-}$ to protect *P. americana* from *H. thompsonii* toxicity, and the SOD activity was adequate to copeup with an increased concentration of such superoxide radicals to prevent it from oxidative damage by converting the superoxide radicals into less toxic H_2O_2 (Fig.1 b). Our results are in agreement with Peric-mataruga *et al*. [34], who also found that SOD activity increases in the midgut of *Lymantria dispar* larvae fed on unfavourable plants. Aslanturk *et al*., [35] reported that SOD activity increased significantly when the insects were exposed to methidathion and suggested that SOD was stimulated by scavenging superoxide radical to protect the insect from methidathion stress. Injection mode of application in midgut, fatbody and hemolymph showed an inhibition of SOD activity. Our results revealed that the SOD activity is not sufficient for scavenging excess $^{\bullet}O2^{-}$which have been accumulated in tissues of *P. americana*. Our results are in agreement with Narenbabu *et al*., [30] who observed that SOD activity decreases in *P. americana* after 4th hour post infection of LC50 of M20 isolate of *M. anisopliae* in comparison with the control, whereas, Casano *et al*. [36] has also reported the decrease in the activity of SOD consequent to increased levels of H_2O_2, which inhibit SOD through the formation of excess hydroxyl radicals.

Changes in the activity of glutathione-dependent enzyme, GPx in the midgut, fatbody and haemolymph of the *P. americana* treated with *H. thompsonii* through different routes signifies the insect preparation for adaptive metabolic response to the increased lipid peroxidation level as observed by Chaurasia *et al*., [37]. Antunes *et al*., [38] have reported that lipid hydroperoxides and hydrogen peroxide are reduced by glutathione-dependent GPx for detoxification. In the present study increased activity of GPx in the heamolymph, fatbody and midgut has been observed, which might be due to scavenging of free radicals produced by *H. thompsonii* in the insect (Fig1 c). Similar results have been recorded by Hyrl *et al*., [39] for fat body and hemolymph of *G. mellonella* larvae exposed to boric acid and Krishnan *et al*., [40] in the midgut tissue of another lepidopteron under various environmental stresses.

6. Conclusion

The present study revealed that the role of innate antioxidant defense system in enhancing resistance or susceptibility against entomopathogenic fungal conidia. This study will provide additional information on the role of ROS and free radicals of oxygen and biochemical changes in the *H. thompsonii* treated cockroaches. The present study will also be helpful in understanding the interactions between the insect defence system and mycotoxin, which might lead to the production of an effective bioinsecticide.

7. Conflict of interest

The authors declare no conflict of interest with respect to this article.

8. Acknowledgements

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9. References

1. Baumholtz MA, Parish LC, Witkowski JA, Nutting WB. The medical importance of cockroaches. International Journal of Dermatology. 1997; 36:90-96.
2. Fotedar R, Shriniwas UB, Banerjee U, Samantray JC, Nayar E, Verman A. Nosocomial infections: cockroaches as possible vectors of drug-resistant Klebsiella. Journal of Hospital Infection. 1991; 18:155-159.
3. Cotton MF, Wasserman E, Pieper CH, Van Tubbergh D, Campbell G, Fang FC *et al.* Invasive disease due to extended spec-trum beta-lactamase-producing *Klebsiellapneumoniae*in a neonatal unit: the possible role of cockroaches. Journal of Hospital Infection. 2000; 44:13-17.
4. Pai HH, Chen WC, Peng CF. Isolation of bacteria with antibiotic resistance from household cockroaches (Periplanetaamericana and Blattellagermanica). Acta Tropica 2005; 93:259-265.
5. Elbanna SM, Elhadidy NM, Semida FM, Abdel RT. Physiological and biochemical effect of entomopathogenic fungus Metarhiziumanisoplia on the 5th instar of Schistocercagregaria (Orthoptera: Acrididae). Journal of Research Environmetal Science Toxicology. 2012; 1(1):7-18.
6. Gabarty A, El-Sonbaty SM, Ibrahim AA. Synergistic effect of gamma radiation and entomopathogenic fungi

Beauveriabassiana and Metarhiziumanisopliae on the humoral immune enzyme response in cotton leaf worm Spodoptera littorals (Boisd). Journal of Biological Science Academic Egypt. 2013; 6(3):1-10.
7. Khan S, Guo L, Maimaiti Y, Mijit M, Qiu O. Entomopathogenic fungi as microbial biocontrol agent. Molecular Plant Breeding 2012; 3(7):63-79.
8. Gillespie JP, Bateman R, Charnley AK. Role of cuticledegrading proteases in the virulence of Metarhizium spp. for the desert locust, Schistocercagregaria. Journal of Invertebrate Pathology. 1998; 71:128-137.
9. McCoy CW. Fungi: Pest control by Hirsutellathompsonii. In: Burges HD (ed) Microbial Control of Insects, Mites and Plant Diseases. Academic Press, New York, 1981, 499-512.
10. Chandler D, Davidson G, Pell JK, Ball BV, Shaw K, Sunderland KD. Fungal biocontrol of acari. Biocontrol Science and Technology 2000; 10:357-384.
11. Ahmad S, Zaman K, MacGill RS, Batcabe JP, Pardini R. Dichlone-induced oxidative stress in amodel insect species, Spodopteraeridania. Archives of Environmental Contamination and Toxicol. 1995; 29:442-448.
12. Damien C, Chantal VH, Pirouz S, Zerimech FH, Laurence J, Jean MH. Cellular impact of metal trace elements in terricolous lichen Diploschistesmuscorum (Scop.) R. Sant-identification of oxidative stress biomarkers. Water Air Soil Pollution 2004; 152:55-69.
13. Pardini RS. Toxicity of oxygen from naturally occurring redox-active pro-oxidants. Archieves of Insect Biochemistry Physiology 1995; 29:101-118.
14. Lee MY, Shin HW. Cadmium-induced changes in antioxidant enzymes from the marine alge Nannochloropsisoculate. Journal of Applied Phycology. 2003; 15:13-19.
15. Pereira GJG, Molian SMG, Lea PJ, Azevedo RA. Activity of antioxidant enzymes in response to cadmium in Crotalaria juncea. Plant Soil 2002; 239:123-132.
16. Francisco J, Roero JR, Francisco B, Belen R, Jauan S, Antonio L *et al.* Reduction of brain antioxidant defense upon treatment with butylatedhydroxyanisole (BHA) and sudan III in Syrian golden hamster. Neurochemistry Research 2000; 25(3):389-393.
17. Rashed MN. Cadmium and lead levels fish (tilapia nilotica) tissuesas biological indicator for lake water pollution. Environmental Monitoring and Assessment 2001; 68:75-89.
18. Baker JR, Neunzig HH. Hirsutellathompsonii as a fungus parasite of the blueberry bud mite. Journal of Economic Entomolology. 1968; 61:1117-1118.
19. McCoy CW, Allan GE, Ignoffo CM, Jacques RP. Entomopathogens in arthropod pest control programs for citrus. In: Microbial Control of Insect Pests: Future strategies in Pest Management Systems. NSF-USDA-University, Florida, Gainsville, 1978.
20. Abbott WS. A method of computing the effectiveness of an insecticide. Journal of Economic Entomology 1925; 18:265-267.
21. Lowry O, Rosenbraugh N, Farr L, Randall R. Protein measurement with the Folin phenol reagent. Journal of Biological Chemistry 1951; 183:265-275.
22. Bergmeyer HU. Methods of enzymatic analysis, Academic Press, New-york 1974, 4381.
23. Beauchamp C, Fridovich I. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Annals of Biochemistry 1971; 44:276-287.
24. Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of giutathione peroxidase. Journal of Laboratory Medicine. 1987; 70:158-165.
25. Fisher RA, Yates F. Satatistical Tables. London, Oliver and Boyed, 1953.
26. Poprawsk TJ, Parker PE, Tsai JH. Laboratory and field evaluation of hypomycetes insect pathogenic fungi for control of Brown citrus aphid (Homoptera: aphididae) Environmental Entomology 1999; 28:315-321.
27. Laurence N, Sandrine R, Alain J, Catherine V, Alain B, Lysiane R. Clofibric acid or diethylmaleate supplemented diet decrease blood pressure in DOCA-salt treated male Sprague Dawley rats relation with liver antioxidant status. Molecular Cell Biochemistry 2000; 213:65-73.
28. Khaper N, Kaur K, Li T, Farahmand F, Singal PK. Antioxidant enzyme gene expression in congestive heart failure following myocardial infarction. Molecular Cell Biochemistry 2003; 251:9-15.
29. Fornazier RF, Ferreira RR, Vitoria AP, Molina SMG, Lea PJ, Azevedo RA. Effects of cadmium on antioxidant enzyme activities in sugar cane. Biological Plant 2002a; 45(1):91-97.
30. Naren Babu M, Ravi Sankar UV, Padmaja V. Insecticidal activity of the entomopathogenic fungus and the strategy of horizontal transmission for control of cockroaches. International Journal of Plant Animal and Environmental Science. 2014, 4(1).
31. Adamski Z, Ziemnicki K, Fila K, Zikic RV, Stajn A. Effects of long-term exposure to fenitrothion on Spodoptera exigua and tenebrio molitorlarval development and antioxidant enzyme activity. Biological letters 2003; 40:43-52.
32. Rajitha K, Savithri G. Dynamics of catalase in the haemolymph of silkworm bombyxmoriduring the progress of fungal pathogen *beauveriabassiana* (bals.) vuill. International Journal of Biomedical and Pharmaceutical Research. 2014; 5(1):62-65.
33. Allen RG, Farmer KJ, Sohal RS. Effect of catalase inactivation on levels of inorganic peroxides, superoxide dismutase, glutathione, oxygen consumption and life span in adult houseflies (Muscadomestica). Journal of Biochemistry. 1983; 216:503-506.
34. Peric-Mataruga V, Blagojevic D, Spasic MB, Ivanovic-Hladni M. Effect of the host plant on the oxidative defence in the midgut of Lymantriadispar L. caterpillars of different population origins. Journal of Insect Physiology. 1997; 43:101-106.
35. Aslanturk A, Kalender S, Uzunhisarcikli M, Kalender Y. Effects of methidathion on antioxidant enzyme activities and malondialdehyde level in midgut tissues of Lymantriadispar (lepidoptera) larvae. Journal of Entomological Research Society. 2011; 13(3):27-38.
36. Casano LM, Gomez LD, Lascano HR, Gonzalez CA, Trippi VS. Inactivation and degradation of Cu/Zn SOD by active oxygen species in wheat chloroplasts exposed to photo oxidative stress. Plant Cell Physiology 1997; 38:433-440.
37. Chaurasia A, Lone Y, Wani O, Gupta US. Effect of certain entomopathogenic fungi on oxidative stress and mortality of Periplanetaamericana. Pesticide Biochemistry Physiology. doi: 10.1016/j.pestbp.2015.09.002
38. Antunes F, Han D, Cadenas E. Relative contributions of heart mitochondria glutathione peroxidase and catalase to H_2O_2 detoxification in in vivo conditions. Free Radical Biology and Medicine 2002; 33:1260-1267.
39. Hyrsl P, Buyukguzel E, Buyukguzel K. The Effects of

boric acid-induced oxidative stress on antioxidant enzymes and survivorship in Galleria mellonella. Archieves of Insect Biochemistry Physiology 2007; 66:23-31.

40. Krishnan N, Kodrik D, Turanlı F, Sehnal F. Stage-specific distribution of oxidative radicals and antioxidant enzymes in the midgut of Leptinotarsadecemlineata. Journal of Insect Physiology. 2007; 53:67-74.