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Research Article

Enzymatic responses in *Oryzaephilus surinamensis* (Coleoptera: Silvanidea) to plant extracts, entomopathogenic fungi and bacterial agents: Implications for pest control

Usama Afzal[1], Farid Asif Shaheen[1], Qurban Ali[2], Asad Aslam[2*], Muhammad Shehzad[1], Humaira Malik[2], Muhammad Kamil Malik[2], Muhammad Faheem Akhtar[2], Muhammad Bilal Bin Iqbal[2], Muhammad Jawad Saleem[2] and Najuf Awais Anjum[3]

1. Department of Entomology, PMAS Arid Agricultural University, Rawalpindi, Pakistan
2. Entomological Research Institute, Ayub Agricultural Research Institute, Faisalabad, Pakistan
3. Entomological Research Substation, Pasrur, Pakistan

**Corresponding author's email: mr.awan2233@gmail.com*

Citation

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Abstract

Fluctuation in enzymes activity is an indicator of immune response to certain stress insect is observing. *Oryzaephilus surinamensis*, commonly known as sawtoothed grain beetle (STGB), is a devastating insect pest of stored products. Chemical-based management strategies for STGB are commonly used but have certain detrimental implication: residual effects on commodities, posing significant health risks, development of insect resistance. In this study, three different biocontrol treatments were evaluated for their virulence against STGB: plant extract (*Brassica juncea*), entomopathogenic fungus (*Beauveria bassiana*), and the entomopathogenic bacterium (*Xenorhabdus nematophila*). In the later part of current research shift in midgut enzymatic activity under these treatments has also been assessed. Results accentuated that corrected mortality percentage of STGB adults was significantly influenced by the bacterium, plant extract, and fungus. Entomopathogenic fungi, *B. bassiana* induced the highest mortality (66.7%), followed by *X. nematophila* (63.3%), while the lowest mortality in STGB was observed corresponding to *B. juncea*. The aforementioned treatments also inhibited a-amylase, lipase and protease activity in a time dependent fashion with peak α-amylase, protease activity on the third day after treatment, with significant declines in enzyme concentration by the fifth and seventh days. While, lipase activity remained almost constant on days 3 and 5, but a significant decline

was detected on day 7 after treatment. The finding demonstrates that biocontrol tactics possess significant potential for managing stored insect pests, offering a economical and environmentally responsible substitute for chemical insecticides.

Keywords: Entomopathogenic fungus; Enzymatic activity; Insect pests of dates; Plant extract; stored product pest**;** Saw tooth beetle; *Xenorhabdhus nematophila*

Introduction

The sawtoothed grain beetle (*Oryzaephilus. surinamensis* L.) is a significant global pest of stored grains viz. different cereals and other packaging goods. Both adult and larval stages infest grains and their derivatives under storage conditions [1, 2]. In addition to grains and flour, this pest also infests dried fruits viz. preserved dried, meat, dates and various processed food harvests, including sugar and biscuits [2, 3]. Infestations have been reported across all varieties of stored rice [4, 5]. It is estimated that STGB infestations causes annual global losses of approximately 70 billion dollars [6]. This economic impact includes up to 10% losses in stored grains, seeds, and cereals, along with 7% to 16% yield losses in wheat crops [7, 8]. Chemical control particularly fumigation treatments (aluminum phosphide tablets or pellets) and other treatments (Methyl Bromide, Carbon tetrachloride and carbon disulfide mixtures) remains the most commonly used for managing insect pests of stored products [9, 10] particularly STGB infestations due to its effectiveness in disrupting the pest's feeding biology [5, 11, 12]. Alternative methods of control have been implemented to reduce the environmental issues [13, 14] and pesticide residues in stored grain commodities [15] i.e dates. These methods involve manipulating the physical environment viz. application of gas like ozone gas, nitrogen gas and other inert gases of the storage facilities to stop the multiplication of insects and other pest while applying severe temperatures also used to keep beetle populations from reaching the point where they cause economic harm to stored products [2, 5]. Botanical insecticides like neem extracts, eucalyptus extracts, oak extract etc and entomopathogenic fungi (EPF) have gained attention as eco-friendly alternatives to synthetic pesticides for proper management of stored grain insects pest [14, 16]. This biocontrol agent's offer reduced environmental risks i.e pesticide residues while maintaining effectiveness against stored product pests [14, 15]. Different researches have been demonstrated the effectiveness of *M. anisopliae* and *B. bassiana* in controlling stored-product insect pests [17]. Entomopathogenic fungi (EPF) given their broad host range for its effective control under control and field conditions [18].

Enzymes play a critical role in various biological procedures in insects, including metabolism, digestion, and detoxification mechanisms against insecticides [5, 19, 20]. Insect can enhance their detoxification processes through the action of digestive enzymes in retort to specific food sources or toxins [17, 21]. Key digestive enzymes such as amylase, lipase, and protease contribute significantly to insect metabolism. α-Amylase is a particularly important hydrolytic enzyme intricate in carbohydrate digestion in grain beetles [11, 22]. Protease plays an important part in insect pest metamorphosis and food breakdown both internally and externally [1, 8, 20]. The impartial of this research was to assess metabolic enzyme activities in STGB following the application of plant extract (*Brassica juncea***),** entomopathogenic fungus (*Beauveria bassiana*), and entomopathogenic bacterium (*Xenorhabdus nematophila*). Understanding the enzymatic response to these treatments will provide insights into the potential use of insect

digestive enzymes as targets for biopesticide development and novel pest management strategies.

Material and Methods

Experimental Conditions

The study was carried out using a complete randomized design (CRD) at the stored grain laboratory at the Entomological Research Institute in Faisalabad. A homogenous population of sawtoothed grain beetle (STGB) was reared on dates under controlled environmental conditions (28 ± 2 °C at a relative humidity of 65 ± 5% with a 16:8 light:dark cycle) .

Plant material

Seed extract of *Brassica juncea* was prepared by following the protocol developed by Azubuike *et al.* **[23]** with slight modifications. The insecticidal activity linked to the previously mentioned plant material has been documented by various researchers therefore, in this study, LC50 was utilized based on preliminary experiments. Briefly, the seeds were cleaned, finely ground in a coffee grinder and kept in a sterile container at room temperature in a dark location. 300 mL of 70% ethanol was used to extract a 100g sample of powdered seeds, which was then left for 72 hours. The dried extract was collected in sterilized 50 ml flasks and stored for experimental use.

Microbial agents

Laboratory reared cultured of *B. bassiana* were used in pathogenicity trials. In 9-cm-diameter Petri dishes, the fungus was grown on potato dextrose agar (PDA). It was then cultured for 15 days at 28°C, 65±5% relative humidity (RH), and a photoperiod of 12:12 (L:D) hours. Twenty milliliters of sterile distilled water containing a 0.05% Tween-80 solution was added in order to harvest conidia. A Neuberger chamber was used to measure the concentration of conidia per milliliter after the conidia suspension was filtered through a 90-µm mesh screen. The LC_{50} concentration (1×10^4 conidia/mL) was calibrated as described by Shehzad *et al.* **[18]**. A stock inoculum of *X. nematophila* was obtained from previously stored bacterial culture. The inoculum was scattered onto nutritional agar (NA) plates and incubated for 48 hours at 28°C to promote the development of new bacteria. In a rotary shaker, liquid cultures were cultivated at 30°C in Luria-Bertani (LB) medium devoid of antibiotics.

Enzymatic assay

Treatments were applied and LC_{50} was calculated. After that insects were homogenized separately in Eppendorf tube and 500 µl of ice cold sodium phosphate buffer (20 mM, pH 7.0) was added in homogenate. The homogenates was centrifuged at 1700rpm for twenty minutes at 4ºC temperature as per protocol given by Koodalingam *et al.* **[24]**. LC50 concentrations of each plant extract, fungus, and bacterium were determined and applied to adult STGB (*Oryzaephilus surinamensis*) following the methodology of Al-Dhaheri& Al-Deeb **[4]**.

Table 1: LC50 concentrations of each plant extract, fungus and bacterium

Concentration	Concentrations used
Brassica juncea (%)	20
Beauveria bassiana (Conidia/ml)	1.7×10^4 **conidia/mL**
Xenorhabdhus nematophila (Cfu/ml)	1.5×10^6

Sample preparation

Eppendorf tubes were used to homogenize STGB people in 500 µL of ice-cold sodium phosphate buffer (20 mM, pH 7.0). The supernatants were taken out for further enzymatic examination after the

homogenates were centrifuged for 20 minutes at 1,000 rpm. The homogenates were kept refrigerated until they were needed, in accordance with Koodalingam *et al*. [24].

Protein concentration

To determine the protein concentration, the Bradford [25] technique was used. Two microliters of the STGB homogenate sample were combined with 400 microliters of the The Coomassie Plus Protein Assay reagent was combined with the mixture, which was then permitted to settle at room temperature for five minutes. The standard used to create the calibration curve was bovine serum albumin (BSA). A spectrophotometer was used to measure the amount of protein present.

α-amylase Activity

This α-amylase activity was measured using Malaikozhundan and Vinodhini's technique [26]. The substrate was a soluble starch solution at 1% (w/v). After adding 6 µL of the starch solution or water to 44 µL of the insect homogenate, the mixture was incubated for 45 minutes at 37°C. At 340 nm, absorbance was measured after 3,5-dinitrosalicylic acid (DNS) was added to stop the process. Using a standard curve of α-amylase absorbance versus the amount of maltose generated, enzyme activity was measured.

Lipase activity

Khosravi and Sendi [27] provided the technique used to assess lipase activity. Before being combined with 18 µL of p-nitrophenyl butyrate substrate, a 10 µL sample of insect extract was diluted with 172 µL of universal buffer solution. After the reaction mixture was incubated at 37°C, absorbance at 405 nm was measured. Enzyme activity was measured as the quantity of enzyme needed to hydrolyse p-nitrophenyl butyrate to p-nitrophenyl at 37°C and pH 7.2.

Protease activity

Azocasein was used as a substrate to measure the protease activity in adult STGB. The reaction mixture included 80 µL of a 2% azocasein solution, 30 µL of enzyme extract, and a universal buffer. 10% trichloroacetic acid was added to stop the reaction after the mixture had been incubated for 60 minutes at 37°C. The mixture was centrifuged at 16,000 rpm for 10 minutes after chilling to 4°C for 120 minutes. After adding 1 N NaOH to the supernatant, absorbance at 450 nm was determined by Khosravi and Sendi [27].

Statistical analysis

Probit analysis was used to get the LC50 values [28, 29]. SPSS 20.0 for Windows was used to conduct the statistical analysis. To compare means, Duncan's Multiple Range Test (DMRT) was used. Microsoft Excel was used to create the graphs.

Results and discussion

This study aimed to evaluate the mortality percentage and enzymatic responses **(***α-amylase, lipase,* and *protease***)** of *O. surinamensis* exposed to *B. juncea, B. bassiana*, and *X. nematophila.* This results and discussion regarding the mortality percentage and enzymatic responses **(***α-amylase, lipase,* and *protease***)** of *O. surinamensis* exposed to *B. juncea, B. bassiana*, and *X. nematophila* are following.

Percentage mortality

Figure 1 illustrates the mortality of *O. surinamensis* adults exposed to bacterial, plant extract, and entomopathogenic fungal treatments. Among the treatments, the fungus exhibited the highest mortality rate, reaching 66.7%. The bacterium also induced significant mortality, with a peak of 63.3%. The plant extract showed a comparatively lower mortality rate of 56.7%. Mortality varied across different time points, suggesting distinct modes of action among the treatments. The plant extracts, bacterium and fungi demonstrated insecticidal properties against sawtooth beetle**.** The

mortality data indicates that fungal and bacterial treatments were more effective than the plant extract**.** Becke *et al.* [30] similarly evaluated plant extracts and microbial agents against mealybugs, finding significant long-term insect control, albeit on a different species. Ismail Asad Aslam, Muhammad Jawad Saleem, Muhammad Kmail Malik and Najaf Awais Anjum [31] also highlighted the insecticidal effects of *B. bassiana* and diatomaceous earth on red flour beetles (*Tribolium castaneum*). Krishna and Yadav [32] concluded that non-chemical insecticides are cost-effective, eco-friendly alternatives for managing stored grain pests like *T. castaneum*.

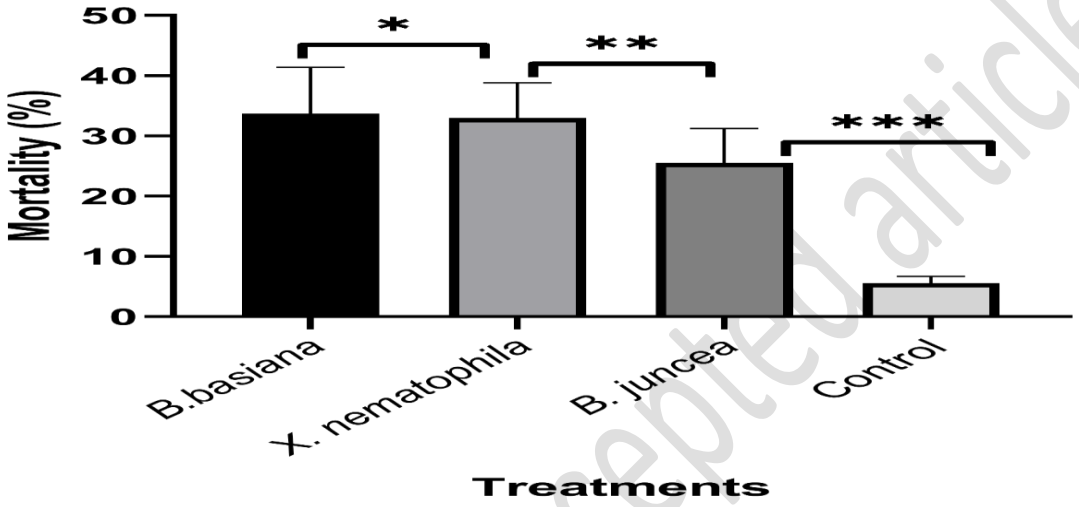

Figure 1: Comparison of *O.surinamensis* mortality in response to plant extracts, Entomopathogenic fungi, and Bacterial agents

α-Amylase* Activity of *O. surinamensis

The highest *α*-amylase activity was observed in beetles treated with *Brassica juncea* on the 3rd day (0.026 units/mg protein), followed by those treated with *Beauveria bassiana* and *Xenorhabdus nematophila* on the 5th day (0.021 units/mg protein). The lowest enzyme concentration was recorded in beetles treated with *X. nematophila* on the 3rd day (0.03 units/mg protein) (Table 2). Enzymes such as amylase, lipase, and protease play crucial roles in insect digestion and adaptation to environmental stress [28, 33]. The results showed that *B. juncea, B. bassiana*, and *X. nematophila* significantly influenced *O. surinamensis* detoxification and digestive enzyme activity, with greater reductions observed over prolonged exposure. The suppression of α-amylase activity at extended exposure times suggests a disruption in metabolic transit rates potentially due to the direct effects of the treatments on enzyme regulation [33]. Among all treatments, *B. juncea* significantly reduced α-amylase activity, likely due to its antifeedant properties [34]. Similar findings were reported by Farias *et al* [35], who observed amylase inhibition from Carica papaya and *Ricinus communis* seed extracts against coleopteran pests.

Table 2. Comparison of *α-amylase* (units/mg protein ± SEM) Activity of *O. surinamensis* exposed to *B. juncea, B. bassiana,* and *X. nematophila* Activity

Treatment	3rd Day	5th Day	7th Day
Brassica juncea	0.026 ± 0.006a	0.19 ± 0.01b	0.019 ± 0.003b
Beauveria bassiana	0.18 ± 0.005b	0.021 ± 0.006ab	0.008 ± 0.001c
Xenorhabdus nematophila	0.03 ± 0.005c	0.021 ± 0.006ab	0.018 ± 0.002b

Lipase Activity of *O. surinamensis*

Lipase activity remained relatively stable during the first five days across all treatments, resulting in a non-significant interaction (Fig. 2). However, significant differences were observed on the 7th day. The lowest lipase activity was recorded in beetles treated with *B. bassiana* (0.03 µmol/min), followed by those treated with *X. nematophila* (0.04 µmol/min). The highest lipase activity was observed in the untreated control group (0.07 µmol/min) on the 7th day. These findings suggest that *B. juncea, B. bassiana,* and *X. nematophila* effectively inhibited lipase activity compared to the control. Plant extracts have been reported to inhibit metabolic enzymes, including lipase [28], amylase [36] and protease [37]. Lipids play an essential role in insect energy metabolism [38] and the observed reduction in lipase activity in treated beetles aligns with findings by Madasamy *et al.* [39]. The decline in lipid content may result from protein conversion or alternative energy production [40]. Similar studies by Nathan *et al.* [41] demonstrated that neem-based insecticides significantly affected lipase activity in *Cnaphalocrocis medinalis*. Additionally, Zibaee *et al.* [42] reported Artemisia annua extract inhibition of lipase activity in *Chilo suppressalis.*

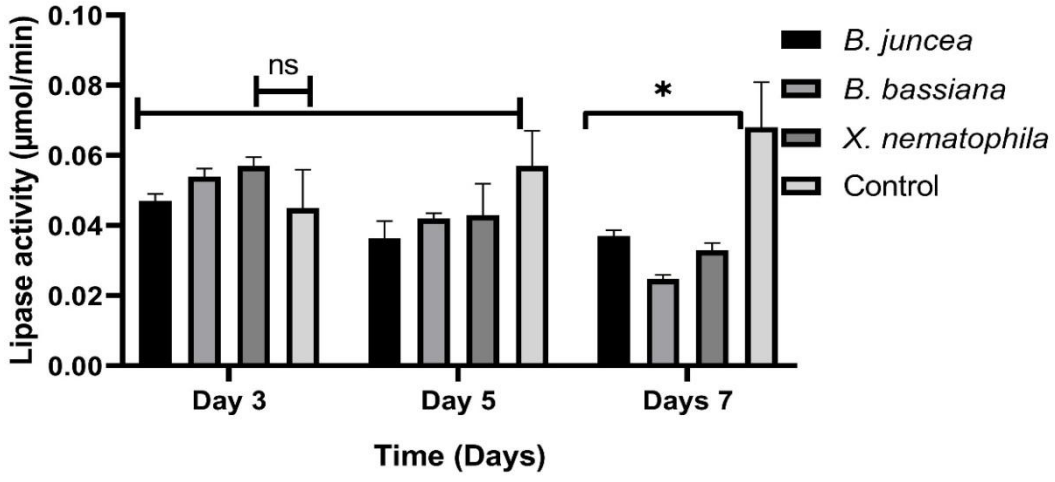

Figure 2: Comparison of Lipase Activity of *O. surinamensis* exposed to *B. juncea, B. bassiana,* and *X. nematophila*

Protease activity of *O. surinamensis*

Significant variations in protease activity were observed across treatments on the 3rd, 5th, and 7th days (Fig. 3). The lowest protease activity was recorded in beetles treated with *B. juncea* on the 3rd (0.17 µmol/min/mg protein) and 5th days (0.16 µmol/min/mg protein). On the 7th day, beetles treated with *X. nematophila* exhibited the lowest protease activity (0.15 µmol/min/mg protein). The control group showed the highest protease activity on the 3rd, 5th, and 7th days (0.5, 0.6, and 0.75 µmol/min, respectively). These results indicate that the tested treatments significantly inhibited protease activity compared to the control. Proteases, including serine, cysteine, and aspartic proteinases, are critical for insect digestion [43]. Some botanical pesticides disrupt protease formation, impairing insect protein metabolism [33, 41]. Antifeedant compounds can inhibit protease activity through direct physiological interactions [39]. Protease suppression has also been observed in *Helicoverpa armigera* treated with nanoparticles [44] and in *Hyphantria cunea* exposed to crude plant extracts [45].

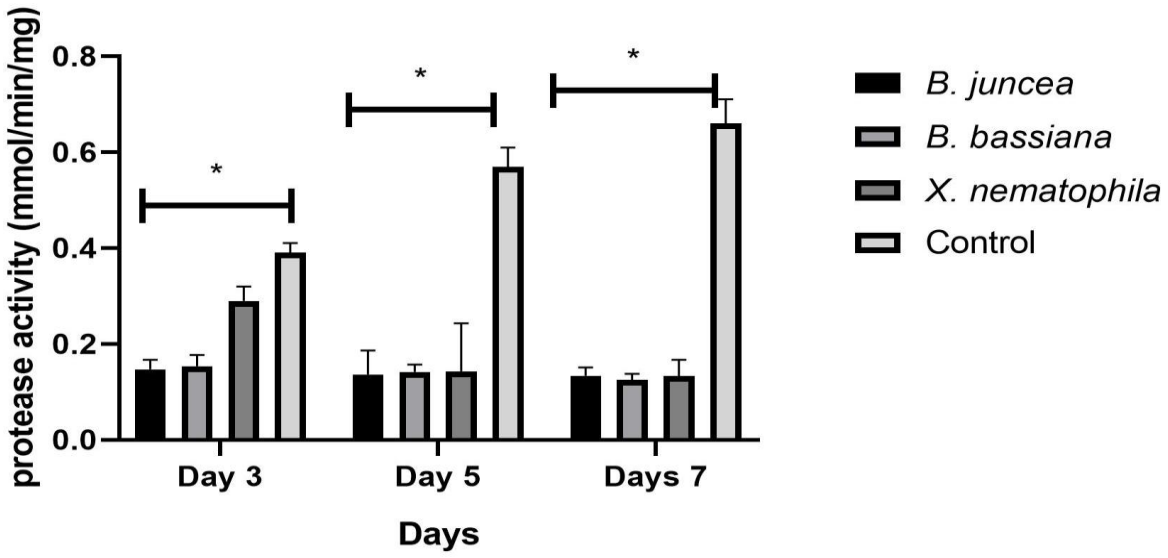

Figure 3: Comparison of Protease Activity of *O. surinamensis* exposed to *B. juncea, B. bassiana,* and *X. nematophila*

Conclusion

Overall, these findings suggest that plant extracts and microbial treatments effectively disrupt digestive enzyme activity in *O. surinamensis*, contributing to their insecticidal properties. These findings emphasize the importance of integrated pest management strategies that combine multiple biocontrol agents to enhance effectiveness while minimizing resistance development in *O. surinamensis.* Further research is needed to elucidate the molecular mechanisms behind these enzymatic disruptions and their potential implications for integrated pest management strategies

Author contributions

Conceived and design the experiment: F A Sheheed, U Afzal, Q Ali & M Shehzad, Performed the experiment: U Afzal & M Shehzad, Analyzed the data: A Aslam, H Malik, MF Akhtar, MBB Iqbal, Contribute reagents/ material/ analysis tool: FA

Shaheen, Wrote the paper: A Aslam, MJ Saleem, MK Malik & NA Anjum.

References

1. Hashem, MY, Ahmed SS, El-Mohandes MA & Gharib MA (2012). Susceptibility of different life stages of saw-toothed grain beetle. *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae) to modified atmospheres enriched with carbon dioxide. *J Stored Prod Res* 48(1): 46-51.
2. Abdel-Banat BBMA, El-Shafie HAF & Boukhwa IA (2023) Screening date cultivars for resistance against the saw-toothed grain beetle, *Oryzaephilus surinamensis* L. (Coleoptera: Silvanidae), *J Stored Prod Res* 102: 102120.
3. Trematerra P, & Throne J (2012). Insect and mite pests of durum wheat. Durum wheat, chemistry and technology, Second Edition. AACC International Inc., St. Paul, MN, USA, 73-83.
4. Al-Dhaheri HA & Al-Deeb MA (2012). Mortality and GST enzyme response of saw-toothed grain beetles, *Oryzaephilus surinamensis* (Coleoptera: Silvanidae) exposed to low insecticide concentrations. *J Entomol* 9(6): 396-402.
5. Kader AA & Hussein AM (2009). Harvesting and Postharvest Handling of Dates. Aleppo: ICARDA.
6. Boxall RA (2001). Post-harvest to Insects-aword Overview. *Int Biodeterior Biodegradation* 48: 137-152.
7. Beckel HD, Lorini I, & Lazzari S (2007). Rearing method of *Oryzaephilus surinamensis* (L.) (Coleoptera, Silvanidae) on various wheat grain granulometry. *Rev Bras Entomol* 51(4): 501-505.
8. Gourgouta M, Morrison III, WR, Hagstrum DW & Athanassiou CG (2023). Saw-toothed grain beetle, *Oryzaephilus surinamensis*, an internationally important stored product pest. *J Stored Prod Res* 104: 102165.
9. Malik H, Ali Q, Murtaza G, Aslam A, Din N, Saleem MJ, Abbas A, Jamil S, Hanif MS, Nazir T, Hanif K, Akhtar MF & Anjum NA (2023). Impact of insect growth regulators on reproduction and life stages of *Tribolium Castaneum* (Coleoptera: Tenebrionidae). *J Global Innov Agric Sci* 11:199-203.
10. Ali Q,, Rasul A, Akhtar MF, Aslam A, Shakir HU, Rehman MA, Naveed K, Rehman HS, Anjum NS & Umar MY (2020). Effect of insect growth regulators on fecundity, fertility and adult emergence of *Tribolium castaneum* (herbst) and *Trogoderma granarium* (everts). *Pak J Agri Sci* 57 (3): 799-805.
11. Moawad SS, & Al-Ghamdi FF (2013). Susceptibility of Some Dry Date Cultivars to Infestation by *Oryzaephilus Surinamensis* (L.) (Coleoptera: Silvanidae). *J Exp Agric Int* 3(3): 651-63.
12. Al-Dhaheri HA, & Al-Deeb M A (2012). Mortality and GST enzyme response of saw-toothed grain beetles, *Oryzaephilus surinamensis* (Coleoptera: Silvanidae) exposed to low insecticide concentrations. *J Entomol* 9(6): 396-402.
13. Malik H, Ali Q, Murtaza G, Aslam A, Din N, Saleem MJ, Abbas A, Jamil S, Hanif MS, Nazir T, Hanif K, Akhtar MF & Anjum NA (2023). Impact of insect growth regulators on reproduction and life stages of *Tribolium Castaneum* (Coleoptera: Tenebrionidae). *J Global Innov Agric Sci* 11:199-203.
14. Ataullah M, Riaz S, Tariq M, Aslam A, Qadir A, Asghar A, Shehzad M, Akhtar MF (2024). In vitro exploration of Entomopathogenic nematodes as potential biocontrol agents of brinjal borer *Leucinodes orbonalis* guenée (Lepidoptera: Crambidae). *Plant Prot* 08(01): 79-85.
15. Malik H, Ali Q, Murtaza, Ahmad M, Akhtar MF, Lalarukh I, Nadeem I, Aziz A, Hamid T , Abbas A, Aslam A & Anjum NA (2021). Puissance of nature's wisdom food grade diatomaceous earth against *Tribolium castaneum* (Herbst); a step towards ecofriendly pest management. *Pak J Agri Sci* 58(2): 539-546.

16. Kanwal S, Muntaha ST, Samreen T, Ali Q, Akhtar MF, Lalarukh I, Anjum NA, Nadeem I, Malik H, Aslam A & Qasim MU (2021). Insecticidal and growth inhibitory impact of different plant extracts on *Tribolium castaneum* (Herbst). *Pak J Agri Sci* 58(3): 993-998.
17. Kader MD, Agarwal AHCLD, Abdelrazik, MD, Sharma PK, Ahmady A & Falcone T (2009). Evaluation of post-thaw DNA integrity of mouse blastocysts after ultrarapid and slow freezing. *Fertil Steril* 91: 5.
18. Shehzad M, Tariq, M, Mukhtar T, & Gulzar A (2021). On the virulence of the entomopathogenic fungi, *Beauveria bassiana* and *Metarhizium anisopliae* (Ascomycota: Hypocreales), against the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae). *Egypt J Biol Pest Co* 31(1): 1-7.
19. Oakeshott J, Claudianos C, Campbell PM, Newcomb RD, & Russell R (2010). Biochemical genetics and genomics of insect esterases. Comprehensive molecular insect science. 5.
20. Ahn JE & Zhu-Salzman K (2009). CmCatD, a cathepsin D-like protease has a potential role in insect defense against a phytocystatin. *J Insect Physiol* 55(8): 678-685.
21. Wang J, Song J, Fang Q, Yao H, Wang F, Song Q & Ye G (2020). Insight into the functional diversification of lipases in the endoparasitoid *Pteromalus puparum* (Hymenoptera: Pteromalidae) by genome-scale annotation and expression analysis. *Insects* 11(4): 227.
22. Al-Deeb MA (2012). Lethal time at different temperatures and date variety preference of the saw-toothed grain beetle in stored dates. *Agric Sci* 3(6): 789-794.
23. Azubuike NC, Okwuosa CN, Maduakor UC, Onwukwe OS, Onyemelukwe AO, Ogu CO & Akande A (2019). Effects of methanolic extract of *Brassica juncea* seeds on biochemical parameters and histological integrity of the heart and liver of albino rats. *Int J Morphol* 37(1): 237-40.
24. Koodalingam A, Mullainadhan P & Arumugam M (2011). Effects of extract of soapnut *Sapindus emarginatus* on esterases and phosphatases of the vector mosquito, *Aedes aegypti* (Diptera: Culicidae). *Acta Tropica* 118(1): 27-36.
25. Bradford M (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Annu Rev Biochem* 72(1): 248-254.
26. Malaikozhundan B & Vinodhini J (2018). Biological control of the Pulse beetle, *Callosobruchus maculatus* in stored grains using the entomopathogenic bacteria, *Bacillus thuringiensis*. *Microb Pathog* 114: 139-146.
27. Khosravi R & Sendi JJ (2013). Effect of neem pesticide (Achook) on midgut enzymatic activities and selected biological compounds in the hemolymph of lesser mulberry pyralid, *Glyphodes pyloalis* Walker (Lepidoptera: Pyralidae *J Plant Prot Res* 5: 238–247.
28. Ogendo JO, Kostyukovsky M, Ravid U, Matasyoh JC, Deng AL, Omolo EO, Pham TA, Kim JJ, Mm, SG, & Kim K (2009). Production of blastospore of entomopathogenic *Beauveria bassiana* in a submerged batch culture. *Mycobiol* 37(3): 218-224.
29. El-Salam EA, Elwahed SA, & Abdel-Rahman RS (2019). Fumigant & toxic activity of some aromatic oils for protecting dry dates from *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae) in stores. *Bull Natl Res Cent* 43(1): 2-6.
30. Becke HI, Achiri TD, Okolle JN, Ntonifor NN, Ruppel S, & Ngosong C (2024). Field efficacy of botanicals and beneficial microbes to control banana mealybug (*Pseudococcus elisae* Borchsenius) (Hemiptera: Pseudococcidae), *Crop Prot* 117.
31. Ismail SM (2025). Residual Efficacy of *Beauveria Bassiana* and Diatomaceous Earth on Wheat Grain against Red Flour

Beetle, *Tribolium Castaneum* (Herbst). *Int J Adv Bio Biomed Res* 13(1): 69-77.

32. Krishna PN & Yadav U (2023). Efficacy of Different Plant Extracts and Insecticide on Red Flour Beetle, *Tribolium castaneum* (Herbst) in Wheat, *Triticum aestivum*. *Int J Environ Clim* 13(9): 508-513.
33. Zibaee A & Bandani AR (2010). Effects of *Artemisia annua* L. (Asteracea) on the digestive enzymatic profiles and the cellular immune reactions of the Sunn pest, *Eurygaster integriceps* (Heteroptera: Scutellaridae), against *Beauveria bassiana*. *Bull Entomol Res* 100: 185–196.
34. Mahey S, Sharma P, Mukerjee SK & Seshadri TR (1972). Structure and synthesis of glabrachromene, a new constituent of *Pongamia glabra*Indian *J Chem* 10: 585.
35. Farias L R, Costa FT, Souza LA, Pelegrini PB, Grossi-de-Sá MF, Neto SM & Franco OL (2007). Isolation of a novel *Carica papaya* αamylase inhibitor with deleterious activity toward *Callosobruchus maculatus*. *Pestic Biochem Physiol* 87(3): 255-260.
36. Franco E, Lubicz V, Mescia F & Tarantino C (2002). Lifetime ratios of beauty hadrons at the next-to-leading order in QCD *Nucl Phys B* 633: 212–236.
37. Nathan SS. & Sehoon K (2006). Effects of *Melia azedarach* (Lin.) extract on the teak *defoliafor Hyblaea* puera (Cramer) (Lepidoptera: Hyblaeidae) *J Crop. Prot* 25: 287–291.
38. Langley KE, Berg TF, Strickland TW, Fenton DM, Boone TC, & Wypych J (1987). Recombinant-DNA-derived bovine growth hormone from Escherichia coli. *Eur J Biochem* 163: 313–321.
39. Madasamy M, Sahayaraj K, Sayed SM, Al-Shuraym LA, Selvaraj P, El-Arnaouty SA & Madasamy K (2023). Insecticidal Mechanism of Botanical Crude Extracts and Their Silver Nanoliquids on *Phenacoccus solenopsis*. *Toxics* 11(4): 305.
40. Xu GF, Wang YY, Han Y, Liu Y, Yang YH, Yu SL, et al. (2015). Growth, feed utilization and body composition of juvenile Manchurian trout, *Brachymystax lenok* (Pallas) fed different dietary protein and lipid levels. *Aquac Nutr* 21: 332-340
41. Nathan SS, Chung PG & Murugan K (2006). Combined effect of biopesticides on the digestive enzymatic profiles of *Cnaphalocrocis medinalis* (Guenee)(the rice leaffolder) (Insecta: Lepidoptera: Pyralidae). *Ecotoxicol Environ Saf* 64(3): 382-389.
42. Zibaee A (2012). A digestive lipase of *Pieris brassicae* L. (Lepidoptera: Pieridae): purification, characterization, and host plants effects. *Arch. Insect Biochem. Physiol* 81(1): 1-19.
43. Terra WR & Ferreira C (2005). 4.5-Biochemistry of Digestion, Editor(s): Lawrence I. Gilbert, Comprehensive Molecular Insect Science, *Elsevier* 171-224.
44. Kantrao S, Ravindra MA, Akbar SMD, Jayanthi PK & Venkataraman A (2017). Effect of biosynthesized Silver nanoparticles on growth and development of *Helicoverpa armigera* (Lepidoptera: Noctuidae): Interaction with midgut protease. *J Asia-Pac Entomol* 20: 583–589.
45. Aghaali N, Ghadamyari M, Hosseininaveh V & Riseh NS (2013) Protease inhibitor from the crude extract of plant seeds affects the digestive proteases in *Hyphantria cunea* (Lep.: Arctiidae) *J Plant Prot Res* 53: 338–346.