Journal of Advanced Zoology

ISSN: 0253-7214

Volume ***45 Special*** *Issue* ***02 Year 2023*** *Page* ***1308:1315***

A SYSTEMIC BIOCHEMICAL STUDY AND QUANTIFICATION ANALYSIS OF SPODOPTERA LITURA AGAINIST PADINA TETRASTROMATICA

***[1]V.Kaviya, , [2]Dr.Sr. Mary Fabiola, [3]V.Ilakkiya**

*[1]Research Scholar, Department of Zoology, Nirmala College for Women, Bharathiyar University, Coimbatore 641018.

[2]Assistant Professor, Department of Zoology, Nirmala College for Women, Bharathiyar University, Coimbatore 641018.

[3]Research Scholar, Department of Zoology, Nirmala College for Women, Bharathiyar University, Coimbatore 641018.

***Corresponding author - kavima26@gmail.com**

Article History

Received: 11 March 2023

Revised: 21 August 2023

Accepted: 03 October 2023

Abstract

Spodoptera litura is an important polyphagous insect pest of several host plants worldwide and has developed resistance to many insecticides. The present study aimed at analysing the effect on a resistant field population from Tamil Nadu, India through biochemical assays. The present study aimed at analysing the effect of selection of a resistant field plant from Sangrur, through synergist and biochemical assays. A screening for larvicidal activity of plant extracts with some known medicinal attributes could lead to the discovery of new agents for pest and vector control. In the backdrop of recent revival of interest in developing plant-based insecticides. In addition to the economic benefits of local use of botanical extracts, the least impact on human health would be enormous, preventing farmers from insecticide poisoning.

Keywords: *Spodoptera litura*; Tamil Nadu; biochemical analysis; larvicidal activity; Pest management.

Introduction

The, *Spodoptera litura*, is a serious polyphagous herbivore, which has caused significant economic damage to cropping systems around the world. As a result, over the past decades large quantities of chemical pesticides have been applied for the control of *S. litura*. However, overuse of synthetic pesticides has resulted in reports of adverse effects, such as pesticide resistance and pest resurgence, and also the contribution to negative impacts on human health and other components of the environment. Due to these reasons, finding alternate control methods including the use of natural control agents is continuing to play an important role in developing management strategies for *S. litura*.

Spodoptera litura (Lepidoptera; Noctuidae) is an important cosmopolitan pest with a wide range of hosts, including different vegetable and field crops such as cotton, groundnut,

soybean, tomato, sweet potato, and tobacco [1,2]. There are numerous reports of the development of resistance in S. litura against a wide range of insecticides, resulting in many sporadic outbreaks of the pests which have led to the failure of crops [3]. Since reports of the presence of this pest in the Hunan province of China, the damage has increased continually. Currently, local farmers mainly depend on synthetic pesticides to control the pest. However, the mismanagement and over-application of synthetic chemicals have caused a high level of resistance to appear in several invertebrate pest populations including *S. litura*.

Biotic and abiotic stresses such as insect attack, pathogen infection, higher or lower temperature, drought and water logging affect the plant growth. Insect pests drastically reduce the crop yield and cause loss of over US$ 14 billion worldwide annually. Furthermore, enormous and indiscriminate use of insecticides causes adverse effect on non-target organisms, pesticide residue in food, pest resurgence, development of insect resistance, toxic effects on human beings and environmental pollution. In this context, host plant resistance is one of the important and eco-friendly approaches of keeping the pest populations below economic injury levels. Improving host plant defence to insects will result in reduced losses due to herbivores, less insecticides use, better crop yields and safer environment.

In this study, the biological impacts of biochemical analysis in *S. litura* were explored. Our results provide further information in support of the combined application of entomopathogenic fungi and natural chemicals in the development of *S.litura* control strategies.

Materials and methods

ESTIMATION PROTEASE ACTIVITY

A stock solution of 1 mg/ml of L-tyrosine was prepared. A calibration line was prepared with the values of absorbance against various working concentrations of L- tyrosine. From this line the actual concentration of produced L-tyrosine was calculated using the experimental absorbance values. Protease activity was assayed by using standard protocol of Faulk et al. For protease enzyme activity, 1 % casein was used as substrate. A mixture of 30 l of crude extract and 600 l of 1% casein was incubated at 37 0C for 30 min. In order to stop the reaction, 270 l of 20% TCA (v/v) were added. The reaction mixture was centrifuged at 5000 rpm for 10 minutes after being stored in an ice bath for 20 minutes to measure absorbance at 280 nm. As the final product, L-tyrosine was generated, and the enzyme activity was calculated as g L-tyrosine released every 30 minutes.

LIPID ESTIMATION BY VANILLIN METHOD

Add 100 ul concentrated sulfuric acid into each tube and incubating at 90 C for 10 min (on a dry heating bath);Cooling to room temperature and measuring background absorbance at 540nm; Prepare the sulfophosphoric vanillin acid agent: 0.2 mg vanillin per ml 17% phosphoric acid) for colour development. Add 50 ul sulfo-phosphoric-vanillin acid agent for colour development; Measuring absorbance at 540 nm after 5 min of colour development.

AGAR- WELL DIFFUSION METHOD

Potato agar medium, 20 ml, was planted in petri dishes. Different sample VK (100 g/ml) was added after wells were cut. The plates were then incubated for 24 hours in a refrigerator. By measuring the diameter of the clearance zone generated around the wells, the protease enzyme activity was assessed. Cover the tubes with Caps on top and incubate at 90^{o} C for 17

minutes or boiling water bath for 10 minutes. Cool to room temperature and measure the optical density at 620 nm against a blank. Prepare a standard curve of absorbance vs glucose.

LIPID ESTIMATION BY VANILLIN METHOD

Add 100 ul concentrated sulfuric acid into each tube and incubating at 90 C for 10 min (on a dry heating bath);Cooling to room temperature and measuring background absorbance at 540nm;Prepare the sulfo-phosphoric-vanillin acid agent: 0.2 mg vanillin per ml 17% phosphoric acid) for color development;Add 50 ulsulfo-phosphoric-vanillin acid agent for color development;Measuring absorbance at 540 nm after 5 min of color development.

Potassium

The flame photometer's filter is set to 766.5 nm (marked for potassium, K), and the flame's colour is adjusted for blueness. The scale is initially set to zero, with the highest standard value as the maximum. The standard solutions are fed to create a standard curve with various concentrations. The sample is injected into the flame photometer after being passed through the filter paper. The concentration is ascertained using a direct reading or the standard curve.

Sodium

The flame photometer's filter is set to 589 nm. The scale is calibrated to zero and maximum using the highest value standard by adding distilled water. By feeding the standard solutions, a standard curve between concentration and emission is created. The sample is put into the flame photometer after being filtered by filter paper, and the concentration is determined either by taking direct measurements or using a graph.

Phosphate

Phosphates can be found in natural or wastewaters as orthophosphates, condensed phosphates, and naturally occurring phosphates. Their presence in water is influenced by biological processes, fertilisers, spent boiler waste, and detergents. They might be discovered in solutions or as detritus. They are both a necessary nutrient for the growth of life and a nutrient that restricts the primary production of the water body. Inorganic phosphorus, one of the most important nutrients for aquatic ecosystems when present in small amounts, can cause algal blooms when paired with nitrates and potassium. Utilizing the stannous chloride method, it is computed.

$$\text{Phosphates (as mg/L)} = \frac{\text{Absorbance of sample } \mathbf{X} \text{ Conc. of Std } \mathbf{X} \text{ 1000}}{\text{Absorbance of Std. } \mathbf{X} \text{ Sample taken}}$$

AGAR- WELL DIFFUSION METHOD

Petri plates containing 20 ml milk agar medium were seeded. Wells were cut and different sample VK (100 µg/ml) was added. The plates were then incubated at refrigerator for 24 hours. The protease enzymatic was assayed by measuring the diameter of the clearances zone formed around the wells.

ANALYSIS OF α-AMYLASE ACTIVITY

It was created a stock solution containing 1 mg/ml of maltose. With the absorbance readings measured against different working maltose concentrations, a calibration line was created. Using the experimental absorbance readings, it was possible to calculate the actual concentration of generated maltose from this line. Using a conventional procedure, the enzyme activity of amylase was assessed. 20 l of glucose solution and 200 l of sodium acetate buffer were combined with 1% glucose as the substrate (0.1M, pH 5.6). The reaction mixture

received 50 l of crude enzyme, and the mixture was incubated at 37 0C for 30 min. 90 l of dinitro salicylic acid solution was added after incubation, and the mixture was then heated for 15 minutes.

RESULTS

Alpha amylase concentration µg/ml in control OD values are 0.881, 0.9, 0.892, Total amylase content µg/ml values as 0.024345, 0.059925, 0.044944 and Mean 0.891. Alpha amylase concentration µg/ml in 500 µg/ml OD values are 1.279, 1.254, 1.342, Total amylase content µg/ml values as 0.769663, 0.722846, 0.88764and Mean 0.793383. Alpha amylase concentration µg/ml in 250µg/ml OD values are 1.208, 1.149, 1.1, Total amylase content µg/ml values as 0.636704, 0.526217, 0.434457 and Mean 0.532459. Alpha amylase concentration µg/ml in 100 µg/ml OD values are 1.076, 1.154, 1.107, Total amylase content µg/ml values as 0.389513, 0.535581, 0.447566 and Mean 0.457553. Alpha amylase concentration µg/ml in 0 µg/ml OD values are 1.047, 1.153, 1.116, Total amylase content µg/ml values as 0.335206, 0.533708, 0.464419 and Mean 0.444444. Alpha amylase concentration µg/ml in 10 µg/ml OD values are 0.621, 0.666, 0.822, Total amylase content µg/ml values as 0and Mean also 0.

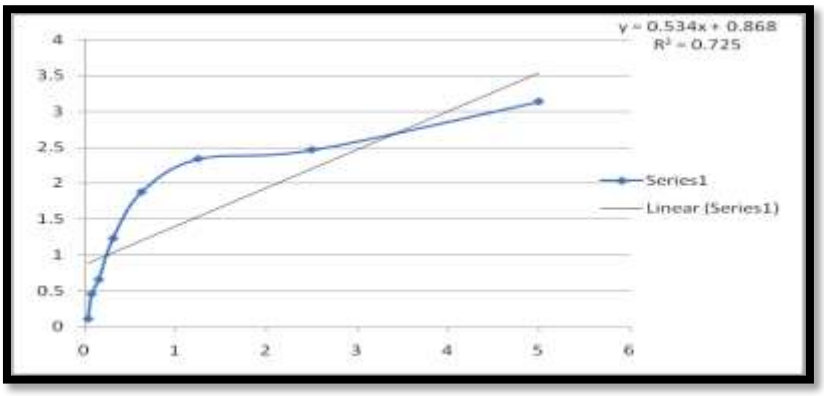

Figure 1: ANALYSIS OF α-AMYLASE ACTIVITY

Considered OD value as 540nm in Triplicates, it contributes in 6 various tested sample concentration (µg/ml), Control contributing values as 0.781, 0.8 and 0.862, 10µg/mlcontributing values as 1.279, 1.254 and 1.342, 50µg/ml contributing values as 1.208, 1.149 and 1.1, 100µg/mlcontributing values as 1.076, 1.154 and 1.107, 250µg/ml contributing values as 1.047, 1.153 and 1.116 and 500µg/ml contributing values as 0.621, 0.666 and 0.882.

ESTIMATION PROTEASE ACTIVITY

Considered OD value as 280nm in Triplicates, it contributes in 6 various tested sample concentration (µg/ml), Control contributing values as 0.781, 0.8 and 0.862, 10µg/mlcontributing values as 1.279, 1.254 and 1.342, 50µg/ml contributing values as 1.208, 1.149 and 1.1, 100µg/ml contributing values as 1.076, 1.154 and 1.107, 250µg/ml contributing values as 1.047, 1.153 and 1.116 and 500µg/ml contributing values as 0.621, 0.666 and 0.882.

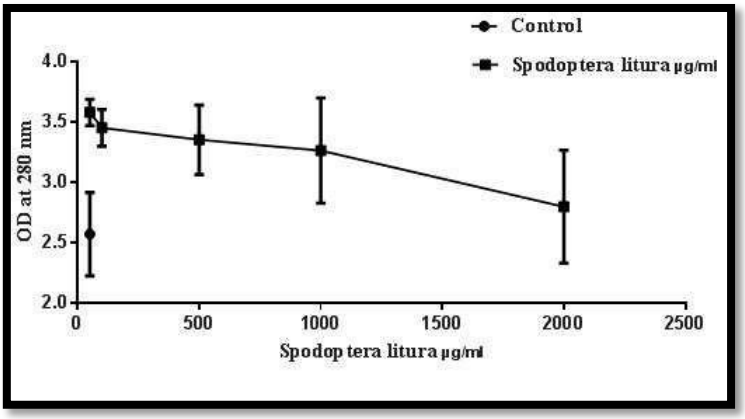

Figure 2:Considered OD value as 280nm

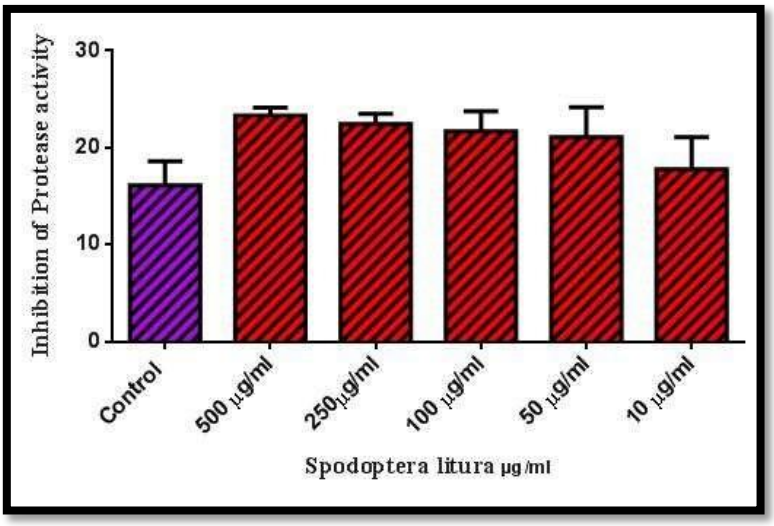

Figure 3: Inhibition of Protease activity

LIPID ESTIMATION BY VANILLIN METHOD

Lipid Calculation 500 µl of standard formulation of chloroform and methanol (2:1) and cholesterol S2 to S8 dilution were used. S1 was also 1ml of stock. Standards for S1 are 10 mg/ml, S2 is 5 mg/ml, S3 is 2.5 mg/ml, S4 is 1.25 mg/ml, S5 is 0.625 mg/ml, S6 is 0.312 mg/ml, S7 is 0.156 mg/ml, and S8 is 0.078 mg/ml.

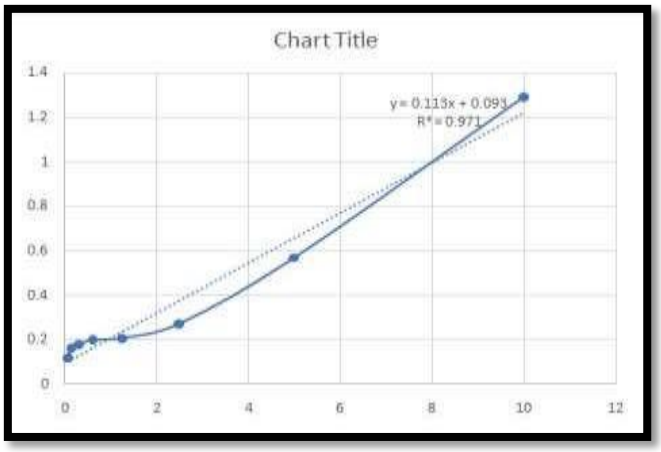

Figure 4:Cholesterol

The Standard Preparation of Cholesterol mg/ml contributes major rise from top to bottom .Conc. of the SampleControl of Mean value of total Lipid content (mg/ml) as 21.97. Mean value of 10µg/ml as 30.87. Mean value of 50µg/ml as 29.72. Mean value of 250µg/ml as 28.38. Mean value of 500µg/ml as 27.32.

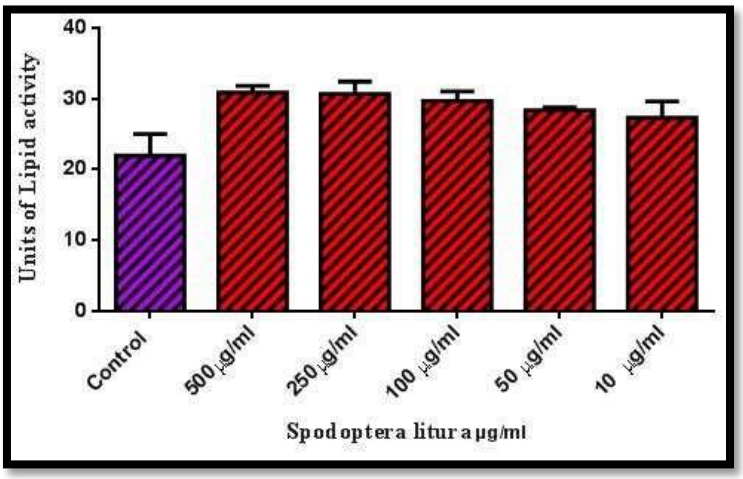

Figure 5: Units of Lipid activity

The total Protein content was present in the extract sample *spodopteralitura*-Control was found to be 21.9712mg/ml.The total Protein content was present in the extract sample *spodoptera litura*-500µg/ml was found to be 30.87mg/ml.The total Protein content was present in the extract sample *spodoptera litura*-250µg/ml was found to be 30.73mg/ml.The total Protein content was present in the extract sample *spodoptera litura*-100µg/ml was found to be 29.72 mg/ml.The total Protein content was present in the extract sample *spodoptera litura*-50µg/ml was found to be 28.38mg/ml.The total Protein content was present in the extract sample *spodoptera litura*-10µg/ml was found to be 27.32 mg/ml.

AGAR- WELL DIFFUSION METHOD

Figure 6: Estimation of carbohydrate by Anthrone method

Mean value of 5 Glucose mg/ml as 2.545. Glucose level of 2.5 as 1.472, Glucose level of 1.25 as 0.978, Glucose level of 0.625 as 0.637, Glucose level of 0.312 as 0.466, Glucose level of 0.156 as 0.385, Glucose level of 0.078 as 0.232, Glucose level of 0.039 as 0.139.

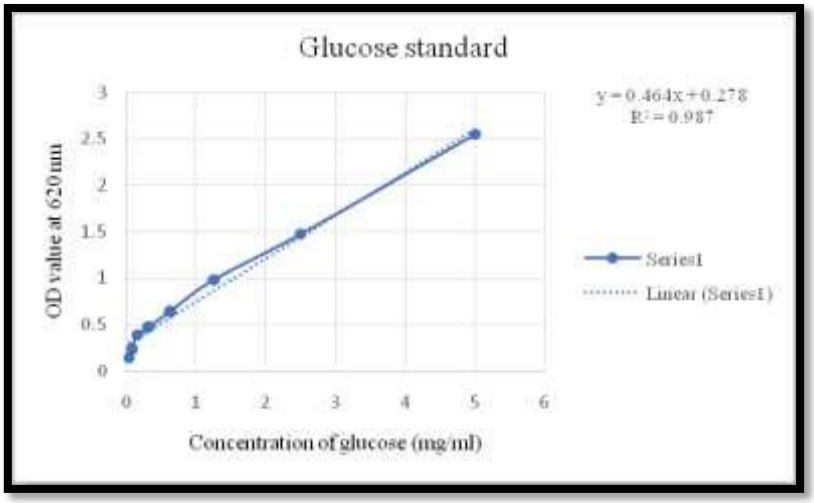

Figure 7:Glucose OD at 620 nm

The Concentration of glucose mg/ml contributes major rise from top to bottom as shown in figure.

LIPID ESTIMATION BY VANILLIN METHOD

Lipid Calculation 500 µl of standard formulation of chloroform and methanol (2:1) and cholesterol S2 to S8 dilution were used. S1 was also 1ml of stock. Standards for S1 are 10 mg/ml, S2 is 5 mg/ml, S3 is 2.5 mg/ml, S4 is 1.25 mg/ml, S5 is 0.625 mg/ml, S6 is 0.312 mg/ml, S7 is 0.156 mg/ml, and S8 is 0.078 mg/ml.

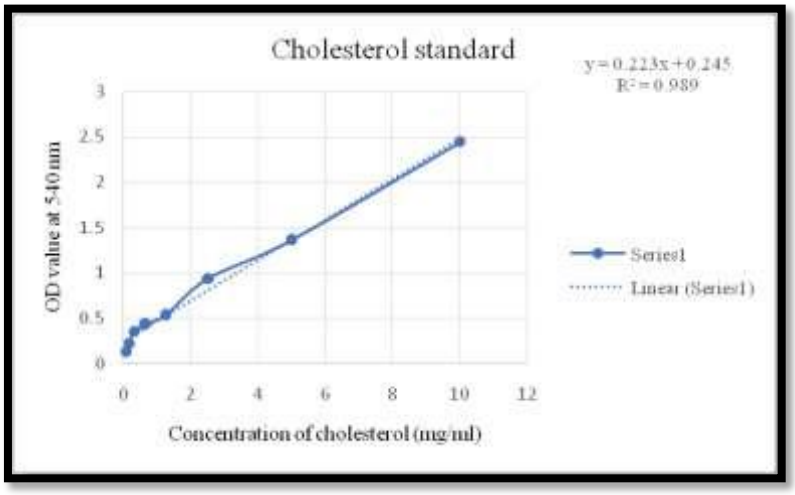

Figure 8:Cholesterol - OD at 540 nm

The Concentration of cholestrol mg/ml contributes major rise from top to bottom as shown in figure SodiumResults of Test sample Mg/L as 1.22. Potassium Results of Test sample Mg/L as 1.05. Phosphate Results of Test sample Mg/L as 0.82.

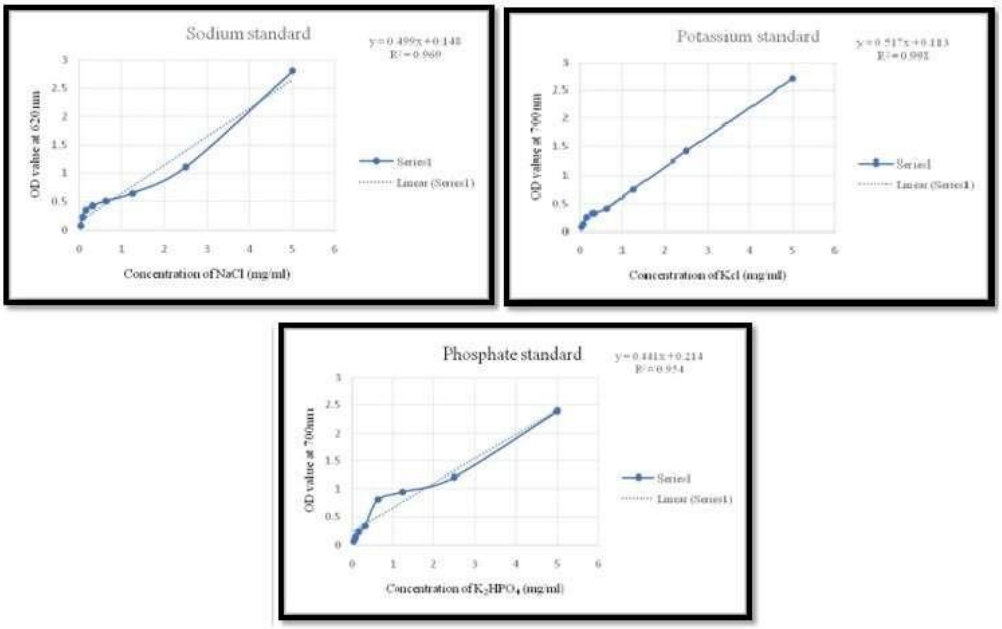

Figure 9: Sodium; potassium and phosphate

Figure 10: Protease enzyme inhibitory assay

CONCLUSION

In summary, this study characterized the bio-insecticidal activity against S. litura but also provides information on the effects of on insect growth parameters as well as the detoxifying mechanism within S. litura. All the above findings suggest that potentially be used within S. litura management programs. However, further work is required to determine the efficacy and persistence under field conditions.

REFERENCES

1. Ahmad M, Mehmood R. 2015. Monitoring of resistance to new chemistry insecticides in *Spodoptera litura* (Lepidoptera: Noctuidae) in Pakistan. Journal of Economic Entomology 108(3): 1279-1288.
2. Ahmad M, Sayyed AH, Saleem MA, Mushtaq A. 2018. Evidence for field evolved resistance to newer insecticides in *Spodoptera litura* (Lepidoptera: Noctuidae) from

Pakistan. Crop Protection 27: 1367-1372.

3. Ahmadi M. 2019. Observed potentiation between pyrethroid and organophosphorus insecticides for the management of *Spodoptera litura* (Lepidoptera: Noctuidae). Crop Protection 28: 264-268.
4. Babu S R, Singh B. 2023. Resistance in *Spodoptera litura* (F.) to insecticides and detoxification enzymes. Indian Journal of Entomology 85(1): 90-94.
5. Ballal C R. 2003. Mass production of *Helicoverpa armigera* and *Spodoptera litura* and their parasitoids. In: Kumari S (Ed.).
6. Booth J, Boyland E, Sims P. 2021. An enzyme from rat liver catalysing conjugations with glutathione Biochemical Journal 79: 516-524.
7. Brown AWA. 1976. How have entomologists dealt with resistance? American Phytopathological Society 3: 67-74.
8. CABI (Centre for Agriculture and Biosciences International).2022. Technical factsheet *Spodoptera litura* (taro caterpillar). CABI Crop Protection Compendium.
9. Che W, Li Y, Zhang D, Qu C, Wang R. 2023. Monitoring and characterization of field-evolved resistance to diamide insecticides in *Spodoptera litura* collected from eastern China. Journal of Applied Entomology.
10. Despres L, David J L, Gallet C. 2017. The evolutionary ecology of insect resistance to plant chemicals. Trends in Ecology & Evolution 22(6): 298-307.
11. Devonshire AL. 2017. The properties of a carboxylesterase from the peach potato aphid, Myzus persicae (Sulz.) and its role in conferring insecticide resistance. Biochemical Journal 167: 675-683.
12. Di HZ, Xia F, Shen LQ, Yu CH, Yu LZ, Fei Y, Pei L, Wu GX. 2014. Biochemical mechanism of chlorantraniliprole resistance in the diamond back moth, *Plutella xylostella*. Journal of integrative agriculture. http://dx.doi.org/10.1016/S2095-3119 (14) 60748-6. accessed April 04, 2020
13. El-Guindy M A, Rehman A, El-Refai M, Abdel-Sattar M M. 2013. The joint action of

mixtures of insecticides, or of insect growth regulators and insecticides, on susceptible and diflubenzuronresistant strains of Spodoptera littoralis Boisd. Journal of Pesticide Science 14: 246-252.

14. Eziah V Y, Rose H A, Clift A D, Mansfield S. 2018. Susceptibility of four field populations of the diamondback moth *Plutella xylostella* (Lepidoptera: Yponomeutidae) to six insecticides in the Sydney region, New South Wales, Australia. Australian Journal of Entomology 47: 355-360.
15. Ferry N, Edward M G, Gatehouse J A, Gatehouse A M. 2004. Plant-insect interactions: molecular approaches to insect resistance. Current Opinion in Biotechnology 15: 155-161.
16. Ffrench-Constant RH, Daborn PJ, Goff GLe. 2014. The genetics and genomics of insecticide resistance. Trends in Genetics 20: 163-170.
17. Goebel R. Jacquemard P. 2020. Evaluation of the sensitivity of the cotton boll pest Heliothis armigera Hbn. to cypermethrin-chlorpyrifos and cypermethrin- methylparathion mixtures: study of possible interactions between these insecticides. Coton et Fibres Tropicales 45: 137-143.
18. Hilliou, F, Chertemps, T, Maïbèche, M, Le Goff G. 2021. Resistance in the genus Spodoptera: Key insect detoxification genes. Insects 12: 544. https://doi.org/10.3390/ insects12060544. accessed June 20, 2023.
19. IRAC Insecticide Resistance Action Committee. 2015. Insecticide Resistance Action Committee, Method No: 007: Leaf eating Lepidoptera and Coleoptera. http://www.irac-online.org/wpcontent/uploads/2009/09/Method007v3june09.pdf, accessed April 09, 2019.
20. Joshi R, Gaur N, Mathpal S. 2022. Distribution of *Spodoptera litura* (F.) in Uttarakhand. Indian Journal of Entomology e22485, 10.55446/ IJE.2022.796, accessed March 13, 2023.
21. Joshi R, Gaur N, Mathpal S. 2023. Insecticide resistance development and detoxification enzyme activities of *Spodoptera litura* (Fabricius) in soybean from Kumaon Himalayas. Legume Research. 10.18805/ LR-5101, accessed June 20, 2023.
22. Kranthi K R. 2005. Insecticide resistance – monitoring, mechanisms and management

manual, Central Institute for Cotton Research.

23. Muthusamy R, Suganya R, Gowri M, Shivakumar M S. 2013. Biochemical mechanisms of organophosphate and pyrethroid resistance in red hairy caterpillar Amsacta albistriga (Lepidoptera: Arctiidae). Journal of the Saudi Society of Agricultural Sciences 12: 47-52.
24. Muthusamy R M, Vishnupriya M S, Shivakumar. 2014. Biochemical mechanism of chlorantraniliprole resistance in *Spodoptera litura* (Fab) (Lepidoptera: Noctuidae). Journal of Asia-Pacific Entomology 17: 865-869.
25. Nehare S, Moharil M P, Ghodki B S, Lande G K, Bisane K D, Thakare A S, Barkhade U P. 2010. Biochemical analysis and synergistic suppression of indoxacarb resistance in *Plutella xylostella*. Journal of Asia Pacific Entomology 13: 91-95.
26. R4P Network. 2016. Trends and challenges in pesticide resistance Detection. Trends in Plant Science 21(10): 834-853.
27. Saleem M, Hussain D, Ghouse G, Abbas M, Fisher S W. 2016. Monitoring of insecticide resistance in *Spodoptera litura* (Lepidoptera:Noctuidae) from four districts of Punjab, Pakistan to conventional and new chemistry insecticides. Crop Protection 79: 177-184.
28. Shen J L, Tan J G, Xiao B, Tan F J, You Z P. 1991. Monitoring and forecasting of *Helicoverpa armigera* (Lepidoptera: Noctuidae) resistance to pyrethroid pesticides in China. Chinese Journal of Applied Entomology 28: 337-341.
29. Shi L, Shi Y, Zhang Y, Liao X. 2019. A systemic study of indoxacarb resistance in *Spodoptera litura* revealed complex expression profles and regulatory mechanism. Scientific Reports 9. https:// doi.org/10.1038/s41598-019-51234-5. accessed June 20, 2023.
30. Singh K N, Sachan G C. 1992. Assessment of yield loss due to insect pests at different growth stages of groundnut in Pantnagar, Uttar Pradesh, India. Crop Protection 11: 414-418.
31. Sreelakshmi P, Mathew T B, Umamaheswaran K, Josephrajkumar A. 2019. Modulation in activity profiles in insecticide-resistant population of tobacco caterpillar, *Spodoptera litura* (Fabricius). Current Science 116(4): 664-669.
32. Su J, Lai T, Li J. 2012. Susceptibility of field populations of *Spodoptera litura* (Fabricius)

(Lepidoptera: Noctuidae) in China to chlorantraniliprole and the activities of detoxification enzymes. Crop Protection 42: 217-222.

33. Tang B, Sun J, Zhou X, Gao X, Liang P. 2011. The stability and biochemical basis of fufenozide resistance in a laboratoryselected strain of *Plutella xylostella*. Pesticide Biochemistry and Physiolology 101: 80-85.
34. Van A K. 1962. A study of housefly esterases by means of a sensitive calorimetric method. Journal of Insect Physiology 8: 401-416.
35. Vengateswari G, Arunthirumeni M, Shivakumar M S. 2020. Effect of food plants on *Spodoptera litura* (Lepidoptera: Noctuidae) larvae immune and antioxidant properties in response to Bacillus thuringiensis infection. Toxicology Reports 7: 1428-1437.
36. Vennila S, Ramamurthy V V, Deshmukh A, Pinjarkar D B, Agarwal M, Pagar P C, Prasad Y G, Prabhakar M, Kranthi K R, Bambawale O M. 2010. A treatise on mealybugs of Central Indian cotton production system. National Centre for Integrated Pest Management, New Delhi, Technical Bulletin 24: 1-39.
37. Wang X, Lou L, Su J. 2019. Prevalence and stability of insecticide resistances in field population of *Spodoptera litura* (Lepidoptera: Noctuidae) from Huizhou, Guangdong Province, China. Journal of Asia-Pacific Entomology 22: 728-732.