INTEGRATED PEST MANAGEMENT STRATEGIES AND ESTERASE ANALYSIS FOR CONTROLLING FALL ARMYWORM INFESTATIONS IN GRAMINAEAE CROPS: A STUDY ON THE ROLE OF TEPHROSIA PURPUREA AND PORTULACA OLERACEA EXTRACTS

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Introduction:

The emergence and spread of agricultural pests pose significant challenges to global food security and crop production. Among these pests, *Spodoptera frugiperda*, commonly known as the fall armyworm, stands out as a highly destructive invader with a broad host range, including crucial crops like maize, rice, and cotton. Its rapid spread and voracious feeding habits have raised alarms, particularly in regions like India, where small-scale farmers heavily rely on these crops for their livelihoods.

Researchers and practitioners are actively studying the fall armyworm to better understand its biology, genetic diversity, and interactions with natural enemies. These efforts emphasize the importance of developing integrated pest management (IPM) strategies that combine biological control methods with conventional approaches. Recent studies focus on various aspects of fall armyworm management, particularly in India, where natural enemies like parasitoids and predators are highlighted as potential biological control agents. Additionally, these studies underscore the genetic similarity between Indian fall armyworm populations and those in other countries, emphasizing the necessity for coordinated global pest management efforts. [1]

Some offer a comprehensive examination of the economic implications and management approaches related to fall armyworm infestations in diverse crops globally. It emphasizes the importance of precise data and tailored management strategies to address the complex challenges posed by this invasive pest. [2]

Roughly few studies explore eco-friendly approaches to managing Spodoptera exigua, advocating for integrated pest management strategies that incorporate

biological, microbial, and cultural controls. Additionally, Tephrosia purpurea, known as Wild Indigo, has been utilized for centuries in traditional medicine and is gaining attention for its potential in agricultural pest control. Its abundance, cost-effectiveness, and eco-friendliness make it a practical choice for farmers, particularly in resource-limited areas. Tephrosia purpurea is seen as a sustainable alternative to chemical pesticides, causing less harm to the environment while effectively managing pests. [3] [4]

Recent studies on Tephrosia purpurea highlight its effectiveness in controlling mosquito populations, attributed to its natural toxin, Rotenone. These studies found that extracts from Tephrosia purpurea can effectively kill mosquito larvae, which transmit diseases like filariasis. Further investigations into the toxicity effects of methanolic extracts from Tephrosia purpurea leaves and stems reveal promising results. These extracts exhibit toxicity against mosquito larvae and induce morphological deformities, indicating their potential as natural pesticides for controlling mosquito populations. Overall, these findings suggest that Tephrosia purpurea extracts could serve as eco-friendly and effective tools in controlling disease spread by mosquitoes. [5] [6].

Considering the growing concern about the environment and the need for safer pest control methods, Tephrosia purpurea seems like a good solution. Our project aims to explore more about the effect of Tephrosia purpurea, which can be used to manage pests sustainably in agriculture and improve public health. Through studying its natural compounds and their mechanism, we hope to contribute to finding better, greener ways to control pests and protect our environment. [7]

Portulaca oleracea, commonly known as purslane, is a versatile plant with a rich history of traditional medicinal uses and culinary applications. In recent years, scientific research has discovered various aspects of this plant, and exploring it has contributed to our understanding of Portulaca oleracea and its diverse range of bioactivities.

Recent studies isolate polysaccharides with antitumor and immunoregulatory activities, suggesting potential applications in cancer treatment and immunotherapy. Phytochemical analyses reveal its antioxidant properties, indicating relevance in functional foods and nutraceuticals. Furthermore, research on its anti-insect properties elucidates mechanisms inhibiting insect larvae growth, contributing to natural pest management strategies and highlighting its ecological significance in agricultural ecosystems.[8] [9] [10] [11]

Collectively, these studies showcase a multidisciplinary approach to exploring the bioactive compounds and agricultural potential of T. purpurea and P. oleracea-related plant species. This research aims to harness their full benefits for therapeutic and agricultural purposes, promoting sustainability and resilience in global food systems.

Objectives:

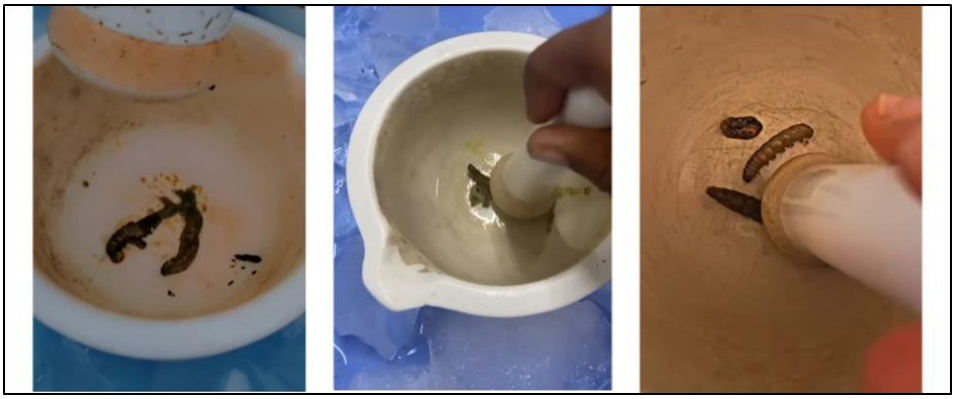
- This study aims to investigate the potential of plant extracts from Tephrosia purpurea, Portulaca oleracea in controlling armyworm infestations.
- Assess the effect of plant extracts on esterase activity in armyworms as a potential mechanism for insect control.
- To reveal insights into the biochemical mechanisms underlying the inhibitory effects of these plant extracts on armyworm.
- To contribute to the development of sustainable pest management strategies and enhance our understanding of enzyme purification for potential industrial applications.
- To explore the effectiveness of natural plant extracts as alternatives to synthetic pesticides for managing armyworm infestations in maize crops.
- Encourage the adoption of eco-friendly pest management strategies that minimize harm to non-target organisms, soil health, and water quality.

Methodology:

TREATMENT OF ARMYWORMS: Armyworm treatment was entailed directly feeding the plants to armyworms, with worms fed maize plants serving as the control. This process continued for 3-4 days.

Experimental feeding of armyworms directly with the Tephrosia purpurea, Portulaca oleracea, and for control, the worms are fed with the maize

LABORATORY ASSAY: Worms treated using direct plant feed were weighed, crushed in a mortar with a pestle under cold conditions, and centrifuged at 10,000rpm at 4°C for 15 minutes. The supernatant was collected, transferred to new vials, and stored at -70°C for further analysis.



Illustrates the picture of crushing the worms treated with the plants separation and analysis

ESTERASE ACTIVITY: Preparation of Substrate Solution: A solution was prepared by diluting α-naphthyl acetate stock in acetone in 0.01M phosphate buffer. Enzyme Preparation: The enzyme stored at -70°C was diluted in a 1:10 ratio using phosphate buffer. Diazoblue-Sodium Lauryl Sulphate Solution: A solution of DBLS was prepared using 1% Diazoblue B and 5% SDS, resulting in a strong red or blue colour. Absorbance measurements were taken at 600nm using a UV-VISIBLE spectrophotometer. Procedure: Enzyme (0.01mL) was mixed with phosphate buffer to make up 1mL, followed by the addition of 1mL of substrate solution and incubation for 15 minutes. After incubation, 1mL of DBLS was added, resulting in an immediate colour change to stable blue, and absorbance was measured at 600nm. [12]

ESTIMATION OF PROTEIN: The protein concentration was determined using the Bradford method (Bradford, 1976). protein samples were mixed with Bradford reagent. Absorbance was measured at 595 nm using a spectrophotometer. Protein concentration was calculated using a standard curve generated with known concentrations of bovine serum albumin (BSA) [13]

SDS PAGE: Analysis of protein pattern was carried out using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The resolving gel and stacking gel, containing 30% acrylamide, underwent electrolysis at 100 V for 70 minutes. Subsequently, the gel was extracted and stained using a staining solution. [14]

NATIVE PAGE (N-PAGE): Native PAGE analysis is performed to assess differences in esterase activity among treatment groups. This technique preserves the native conformation and charge of proteins, providing insights into their functional properties and any alterations caused by the treatments. [15]

PURIFICATION AND CHARACTERIZATION: The purification process of the esterase enzyme involved ion exchange chromatography, specifically using DEAE Cellulose and Sephadex G200. DEAE Cellulose was activated by soaking it in distilled water overnight, followed by a series of washes with acid and base followed by equilibration with phosphate buffer. The sample was then loaded onto the column, and fractions were collected, including flow-through and wash fractions. Bound proteins were eluted stepwise with increasing concentrations of NaCl, and fractions

were collected for analysis. Esterase activity was assessed in all fractions, and a graph was plotted to visualize the distribution of activity. Overall, the purification process successfully purifies the esterase enzyme, as indicated by the analysis of fractions for esterase activity. [16] This purification process separates the enzyme from other proteins and contaminants, allowing for further characterization. The purified enzyme is then characterized by determining its kinetic properties, such as the Michaelis constant (Km) and maximum velocity (Vmax). The activity of the esterase enzyme was measured using a spectrophotometric assay with α-naphthol acetate and methyl gallate as the substrates. The absorbance of samples was recorded at specific wavelengths to determine enzyme activity. The enzyme's substrate specificity was examined to understand which substrate it can cleave. This involved testing the enzyme activity with various substrate concentrations. The graph was plotted by taking 1/[S] and 1/[V] on the X-axis and Y-axis respectively. [17]

COMPARATIVE ANALYSIS OF PLANT-FED ARMYWORM AND SYNTHETIC PESTICIDES: In this study, we conducted a comparative analysis between plant fed armyworms and pesticide treated to assess their efficacy in inhibiting armyworms. Specifically, we examined the effects of Portulaca oleracea-fed armyworm extract as a potential natural alternative to synthetic pesticides. The experiment involved subjecting armyworms to both the plant extract and the synthetic pesticide, observing and measuring their inhibitory effects.

Conclusion:

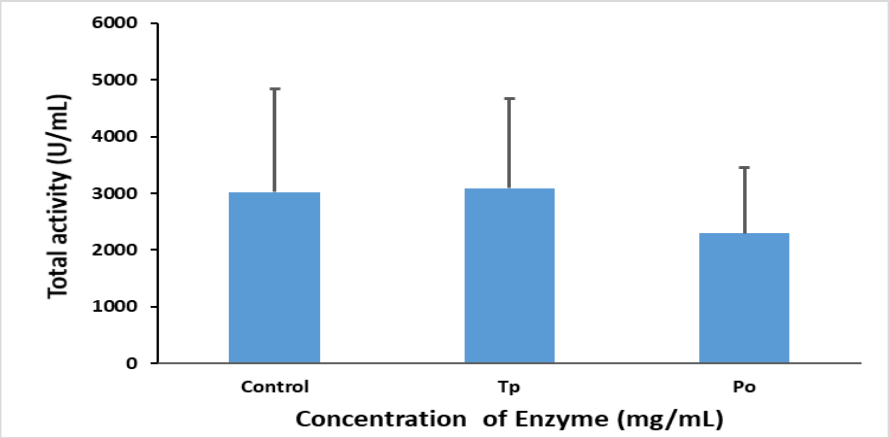
ESTERASE ASSAY: The esterase activity was measured using a spectrophotometric assay with α-naphthol acetate as the substrate.

Illustrates the total activity of esterase in control, *Tephrosia purpurea, Portulaca oleracea* treated worm extracts

The esterase activity in the Tephrosia purpurea treated worm extracts was higher compared to the control, Portulaca oleracea, indicating the presence of an active esterase enzyme. The lower esterase activity is a significant result, that shows that the Portulaca oleracea shows an inhibitory effect on the enzyme activity.

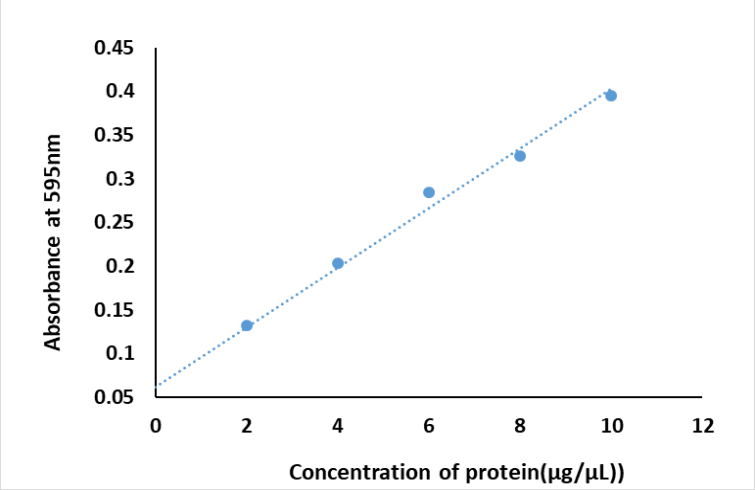
	Trail 1 Total Activity (U/mL)	Trail 2 Total Activity (U/mL)	Trail 3 Total Activity (U/mL)	Average Total Activity (U/mL)	std. deviation
Control	4976.2	2706.6	1375.1	3019.3	1820.800997
T. purpurea	4505.6	3367.7	1393.3	3088.86	4174.355101
P. oleracea	3389.7	2403.3	1085.7	292.9	663.243354

Table 1: Illustrates the total activity of esterase in control, Tephrosia purpurea, Portulaca oleracea treated worm extracts



Graph 1: Illustrates the graph of esterase total activity in armyworms directly treated with control, Tephrosia purpurea, Portulaca oleracea plants

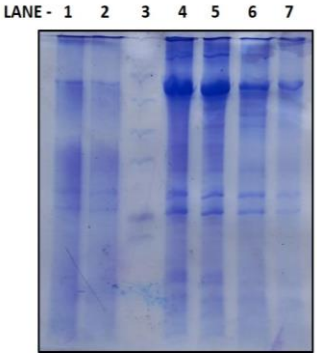
BRADFORD ASSAY:



Graph 2: Illustrates the graph of the standard curve of protein

By the standard curve, we determined: Protein concentration of control: 0.6µg/µl
Protein concentration of Tp: 0.2µg/µL
Protein concentration of Po: 1.6µg/µL

SDS-PAGE:



lane 1 & lane 2 - Portulaca oleracea

lane 3 – Pre - stained protein ladder

lane 4 & lane 5 - Tephrosia purpurea

lane 6 & lane 7- Control

Protein profile on SDS-PAGE

The SDS-PAGE analysis revealed that, the protein degradation is observed in the Portulaca oleracea treated armyworms sample compared to the control and Tephrosia purpurea. the Portulaca oleracea plant appears to have maximum effect on the armyworms where Tephrosia purpurea shows minimal effect.

NATIVE PAGE:

lane 1 - control

lane 2 - Tephrosia purpurea

lane 3 - Portulaca oleracea

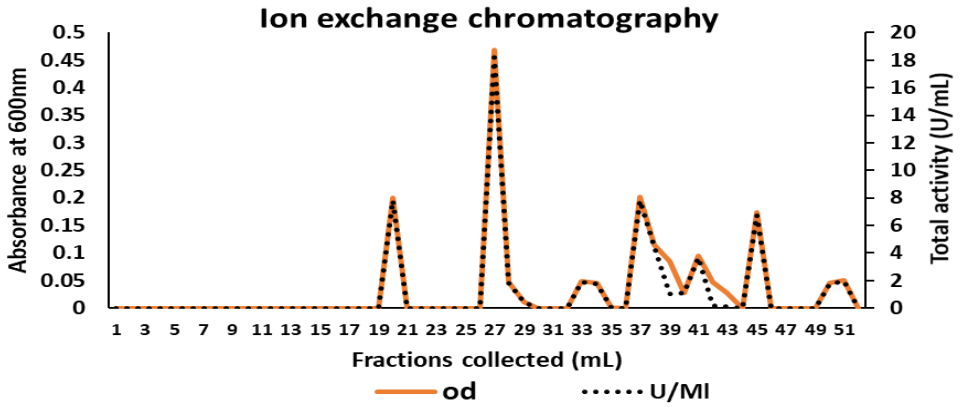
Native PAGE analysis of esterase activity in armyworms treated with plant extracts

These bands displayed varying intensities and migration patterns across the different gel percentages. However, in the Portulaca-treated sample, esterase activity was lower compared to both the control and Tephrosia-treated samples. It shows potential inhibitory effect of Portulaca oleracea extract on esterase enzyme activity in the armyworm.

ION EXCHANGE CHROMATOGRAPHY:

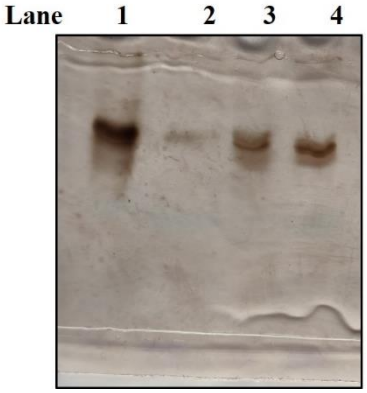
Elution of Esterase Enzyme from DEAE Cellulose

In the Ion exchange chromatography of esterase activity, five distinct peaks were observed. peak 1 eluted at the 100mM of Nacl, peak 2 eluted at the 200mM of Nacl, peak 3 eluted at the 400mM of Nacl, peak 4 eluted at the 400mM of Nacl, peak 5 eluted at the 500mM of Nacl.



Elution profile of Esterase Enzyme from DEAE Cellulose

The presence of multiple peaks suggests the presence of different esterase isoforms or substrates with varying affinities for the ion exchange column. The observed peaks may correspond to different enzymatic forms or substrate specificities within the sampl.



lane 1- control

lane 2- control + pesticide

lane 3- Portulaca oleracea

lane 4- Tephrosia purpurea

Comparative Analysis of plant-Fed Armyworm Extract and Synthetic Pesticide

The results revealed that the synthetic pesticide control exhibited a higher level of inhibition against armyworm infestations compared to the tephrosia-fed armyworm extract. While both treatments demonstrated inhibitory effects. However, it is noteworthy that the Portulaca oleracea-fed armyworm extract displayed significant inhibition. Understanding how different ingredients interact could help us create more powerful bug-fighting formulas. The purified compounds from plants will display a better inhibitory effect than direct plants. Portulaca oleracea extract shows promise as a potential alternative for eco-friendly pest management strategies.

Scope for future work:

Scope for future work in this research project provides exciting opportunities for further investigation and improvement. Firstly, we can look into better ways of getting strong bug-killing substances from Tephrosia purpurea and Portulaca oleracea by trying different methods and parts of the plants. Secondly, we can explore how these

plant extracts might work even better when combined or mixed with other natural or man-made chemicals. Understanding how different ingredients interact could help us create more powerful bug-fighting formulas. We also need to see how using plant-based bug control methods might affect things like soil health, how well crops grow, and the balance of nature in the long run. Studying this over time will help us figure out if these methods are good for the environment and if they can be used widely. Moreover, we can test if these plant extracts can fit into bigger plans for managing pests, which include lots of different ways of dealing with bugs. By trying them out in real fields, we can see if they work well, are affordable for farmers, and if farmers like using them. Additionally, we should keep learning about how these plant extracts stop bugs from growing and spreading. Understanding this at a molecular level can help us make better bug-control methods that are more specific and don't harm other living things as much. Over all, the future of this research is exciting and can lead to better ways of controlling pests in agriculture that are good for the environment and for farmers.

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