Executive Summary

Project Title: **Development of biochemical assay as diagnostic tool for synthetic pyrethroid resistance in cattle tick *Hyalomma anatolicum anatolicum***

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Total grant allocated (Rs.) : 9,97,800/-
Total grant received (Rs.) : 9,13,000/-
Final expenditure (Rs.) : 9,12,720/-

Objectives of the project:

a. Detection of synthetic pyrethroid resistance in *Hyalomma anatolicum anatolicum* tick collected from different agro-climatic zones of Punjab.
b. Development of accurate and efficient biochemical assay for the detection of synthetic pyrethroid resistance in *Hyalomma anatolicum anatolicum*.

Summary of the findings

Ticks and the diseases they transmit are a major constraint to livestock production and management in many parts of the world. Losses attributable to ticks are caused either directly through tick worry, blood loss, damage to hides and udders and the injection of toxins, or indirectly through mortality or debility caused by the diseases transmitted by or associated with the ticks.

The resistance status against the most commonly used synthetic pyrethroids (cypermethrin and deltamethrin) in fourteen different isolates of *H. a. anatolicum* collected from the central plain zone (CPZ) and western zone (WZ) of Punjab was determined using FAO larval packet test. In the CPZ, the RR_{50} values ranged from 0.09 to 0.51 and 1.71 to 5.59 for cypermethrin and deltamethrin, respectively whereas RR_{95} values ranged from 0.37 to 1.29 and 2.42 to 33.88 for cypermethrin and deltamethrin, respectively. Five isolates viz., Amritsar, Fathgarh Sahib, Jalandhar, Kapurthala and Patiala were detected as resistant at level I with RR_{50} ranging from 1.71 to 4.91 and resistance level II was detected in Ludhiana isolate (RR_{50}= 5.59) against deltamethrin. But no resistance status was detected against cypermethrin in all isolates of *H. a. anatolicum* collected from central plain zone, Punjab.

In case of WZ, the RR_{50} values ranged from 1.98 to 13.8 and 1.60 to 12.73 for cypermethrin and deltamethrin, respectively whereas RR_{95} values ranged from 0.80 to 6.91 and 2.06 to 6.42 for cypermethrin and deltamethrin, respectively. The characteristics of the dose-response regression lines of all field populations were significantly different from those of the susceptible strain at the 0.05 level. Level II resistance was recorded in Bathinda (RR_{50}=13.8), Mansa (RR_{50}= 5.18) and Sangrur (RR_{95}=6.91) against cypermethrin. Whereas, level II resistance was more pronounced in isolates from Barnala, Bathinda, Mansa and Moga with RR_{50} ranging

from (5.65 to 12.73) and with RR_{95} in isolates from Bathinda (6.42) and Mansa (5.26) level II resistance was recorded against deltamethrin.

Quantitative analysis of general esterase activity (measured by the production of the metabolite naphthol) revealed a range of 4.210 ± 0.46 to 6.056 ± 0.55 and 2.232 ± 0.23 to 2.296 ± 0.20 µmol/min/mg protein for α and β-esterase activity, respectively in different field isolates of *H. a. anatolicum* from CPZ. The highest α and β-esterase activity was observed in Ludhiana isolate. A correlation coefficient (r) of 0.7534 and 0.818 was recorded between RR_{50} for deltamethrin and α and β-esterase activities, respectively and r values of 0.909 and 0.809 were recorded between RR_{95} for deltamethrin and α and β-esterase activities, respectively indicating positive correlation between the deltamethrin resistance and α and β-esterase activity.

However, *H. a. anatolicum* collected from WZ revealed a range of 3.249 ± 0.31 to 4.523 ± 0.59 and 1.747 ± 0.11 to 2.296 ± 0.20 µmol/min/mg protein for α and β-esterase activity, respectively. The highest α-esterase activity was found in Bathinda and β-esterase activity was observed in Barnala isolate. A correlation coefficient (r) between RR_{50} was found to be 0.6718 against cypermethrin and α-esterase activity and 0.7162 against deltamethrin and α- esterase activity, respectively and 0.7839 and 0.6847 were recorded between RR_{95} against deltamethrin and α and β-esterase activities, respectively.

Quantitative analysis of GST activity revealed a range of 0.013128 ± 0.001015 to 0.0301 ± 0.002102 mmol of product/min/mg protein, in different field isolates of *H. a. anatolicum* collected from CPZ. Significantly elevated levels of GST were detected in Fatehgarh Sahib and Ludhiana isolate whereas, highest enzyme activity was recorded in Ludhiana isolate. A correlation coefficient (r) of 0.736 and 0.946 were recorded between deltamethrin RRs (RR_{50} and RR_{95}) and GST activity of various isolates thus indicating correlation between deltamethrin resistance and GST activity. Whereas, *H. a. anatolicum* collected from WZ revealed GST activity in range of 0.0127 ± 0.0007 to 0.0289 ± 0.0017 mmol of product/min/mg protein. Significant elevated levels of GST were detected in Bathinda and Mansa isolate. A correlation coefficient (r) of 0.942 was recorded between RR_{50} against cypermethrin and GST activity of the isolates. Whereas, correlation coefficient (r) of 0.897 and 0.826, respectively were recorded between deltamethrin RRs (RR_{50} and RR_{95}) and GST activities of the isolates.

Gel profiling of the larval tick isolates revealed over-expression and enhanced activity of carboxyl-esterases and acetylcholine esterases enzymes as the bands were found to be more intense in resistant tick isolates indicating increased production associated to SPs resistance. The total mRNA was isolated from all isolates; cDNA was prepared and used as template for the amplification of carboxylesterase gene. Gel electrophoresis of the PCR products in 1.5% ethidium bromide stained agarose gel revealed an amplicon of 372 bp thus confirming the amplification of the gene. Upon the restriction enzyme digestion by EcoR1 a single product of 372bp was recorded, thus indicating the absence of the point mutation reported earlier.

The resistance recorded in tick isolates of central plain zone, Punjab is relatively less pronounced to cypermethrin as compared to deltamethrin at present. The resistance level of I and II was found to be very common in the tick isolates of western zone, Punjab. Selection for acaricide resistance in tick populations is a major consequence of using chemical acaricides widely and frequently. To reduce the utilization of these chemical acaricides with reduced

efficacies other alternative approaches need to be explored involving the use of eco-friendly sustainable methods in a strategic integrated manner. Along with use of hosts with natural resistance to ticks, use of biological control agents and tick vaccines, exploration of the possibilities of using phyto-chemical acaricides has been identified as the future options.

Conclusion: In central plain zone, deltamethrin resistance is prevalent among *H. a. anatolicum* whereas, for cypermethrin they show susceptible status. In western zone, Bathinda, Mansa, and Sangrur isolates showed level II resistant against cypermethrin and deltamethrin resistance was of much higher magnitude. Although, no significant increase in α and β esterase activity levels were recorded but positive correlation between β esterase levels and deltamethrin resistance was recorded which indicates its role in development of resistance. Also, elevated levels of GST were found to be correlated with deltamethrin resistance in *H. a. anatolicum*. Gel Profiling revealed over-expression and enhanced activity of CaEs and AChEs enzymes indicating increased production associated to SPs resistance.

Contribution to the society: The esterase based biochemical assay developed for the early detection of resistance would be of immense help in effective tick control and rationale use of acaricides thus, leading to decrease environmental pollution and residue in milk and milk products.