

## **ABSTRACT**

Rice and wheat are essential crops for food as well as economy of a country. Being an agricultural country for Pakistan, these two crops have contributions in GDP as well. One of the various factors affecting the production of these crops is insect pests. Effective pest management practices require correct and timely identification of insect pest and their natural enemies. Traditional morphological species identification is a complex and time consuming process which requires great taxonomic expertise. However, due to various constraints of traditional taxonomic practices, some new molecular tools including DNA barcoding are being used for species level identification.

The current study was designed to evaluate the authenticity of morphological identification of insect pests and their natural enemies of rice-wheat ecosystem, using DNA barcoding. Insects were collected using sweep net and handpicking methods from rice-wheat ecosystems of districts Lahore, Mandi Bahauddin and Sialkot of Province Punjab, Pakistan. Field collected specimens of insect pests and predators (Spider and non-spider) were brought to laboratory in the Department of Zoology, Government College University Lahore and after washing the specimens were preserved in 95% ethanol and stored at -20°C for further molecular analysis. All the specimens were taxonomically identified by the help of available morphological keys and catalogues. DNA from the left leg of insects and spiders was extracted by using Thermo Scientific GeneJET DNA Purification kit following the company specified protocols. MtCOI barcode marker of each specimen was amplified through PCR. After verification through gel electrophoresis, PCR products were sent to Canadian Centre for Biodiversity Genomics, University of Guelph, Canada, for sequencing.

A total of 5456 insect pests, representing 34 species, 29 genera and 16 families, 2072 non-spider arthropod predator specimens belonging to 15 families, 19 genera and 20 species and 2736 specimens of spiders representing 38 species, 22 genera and 8 families were morphologically identified. Molecular identification also confirmed the same no of species, genera and families for insect pests and non-spider predators. However, spiders molecular identification confirmed presence of 40 genera. Overall, estimated accuracy of morphological identification for all three groups was approximately 90%. A clear gap was observed between conspecific and congeneric species for spider and non-spider predators as well as for insect pests.



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It can be concluded from current study that traditional taxonomic identification of species through morphological keys work convincingly. However, combination of traditional morphology and molecular methods like DNA barcoding can magnify the reliability and accuracy of the results. Overall, current study contributes paramount information to the molecular ecology of economically important insect pests and their natural enemies attacking rice-wheat ecosystems of Punjab, Pakistan. Furthermore, DNA barcode technology will help in developing effective pest management tools for regulating the important pests of agricultural crops.

### **Keywords**

Pest management, DNA barcoding, gel electrophoresis, spider and non-spider predators, DNA purification.