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MOLECULAR IDENTIFICATION OF FIVE PESTS FOUND IN STORED GRAINS BASED ON *MT*COI GENE SEQUENCES IN SOUTH IRAQ

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SUMMARY

The following study investigated the molecular identification of five species of pests that infect stored grains, held from January 2021 to December 2022 in the Misan Province, Southern Iraq. Infected stored grains' samples came from the General Company for grain trading in its three branches, local and central markets, and homes. Results of the presented study showed five species of pests belonging to the order Coleoptera. These species are *Tribolium castaneum*, *Trogoderma granarium, Rhyzopertha dominica, Callosobruchus maculatus,* and *Oryzaephilus mercator*. Using the molecular technique of polymerase chain reaction helped confirm the morphological identification by employing the gene mtCOXI and identify the mutations present in the local species by comparing with the species found in the NCBI. Results of the genetic analysis revealed differences in the sequences of the nitrogenous bases of the gene mtCOXI between the local species and the species recorded in the NCBI. Likewise, variances appeared with the tree drawn between the studied species and the standard samples. The five species of pests also received accession numbers OR343196, OR343199, OR313198, OR343194, and OR343197, respectively, as recorded in the NCBI.

Keywords: mtCOXI, stored grain pests, molecular identification, NCBI, Misan Province, Iraq

Key findings: Polymerase chain reaction technology (PCR) is an excellent technique for diagnosing insect pests that affect stored grains. It can also be effective to confirm species identification when a phenotypic similarity occurs between two species, such that, it is difficult to distinguish between them.

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INTRODUCTION

Cereals, in general, are valuable in global agriculture (Jagshoran and Tripathi, 2004). Stored grains, as exposed during storage to many pests, acquire quantitative and qualitative losses. According to the Food and Agriculture Organization (FAO) of the United Nations, the causes of losses are mainly due to insects, microorganisms, and rodents (Pimentel, 1991). More than 200 species of insect pests infect stored grains and their products (Rajendran and Sriranjini, 2008).

Beetles constitute the vast majority of these pests, belonging to the order Coleoptera, and are responsible for the largest percentage of losses that occur in stored grains (Kumar and Kalita, 2017). The morphological diagnosis of species based on each species' external characteristics necessitates understanding their evolutionary relationships and diversity. Morphological traits have traditionally served to diagnose insect pests (Jinbo *et al.,* 2011).

However, in some cases, this type of diagnosis is longer and more difficult because of the changes occurring in the external characters, as a result of variations arising in environmental and climatic conditions (Barrett and Hebert, 2005). Moreover, the use of taxonomic keys by researchers requires experience (Ball and Armstrong, 2006). In this case, the molecular detection becomes the confirming partial of the morphological detection (Ball *et al.,* 2005; Timm *et al.,* 2007, 2008).

Molecular detection depends on the analysis of genetic material, and this method is efficient and effective in knowing a wide range of species, even when not diagnosing the species or is previously unknown (Rugman-Jones *et al*., 2009). This study is the first in Misan Province, but many studies in Basrah Province have already focused on the genetic material in molecular diagnosis. Kalaf (2021) studied the genetic variation existing between two very similar species, making it difficult to distinguish between them. These two species are *Tribolium castaneum* and *Tribolium confusum*.

Al-farhany and Kareem (2022) studied the genetic diagnosis of five species of the family Muscidae in Basrah, South Iraq. The first-ever study in Misan Province, Southern Iraq, aimed to determine the molecular identification of five species of pests infecting stored grains.

MATERIALS AND METHODS

Collect infected stored grains' samples

The collection of infected stored grains' samples transpired from the General Company for Grain Trade with its three branches in Misan Province and from local and central markets and homes from January 2021 to December 2022. The collected samples' preservation continued in glass bottles to make permanent colonies of obtained pests infecting the stored grains**.** Species detection morphologically relied on the taxonomic keys, with similar species isolated for molecular identification to confirm the morphological detection.

DNA extraction

The isolation of adults for each species occurred separately, and their crushing used a ceramic mortar to obtain a homogeneous powder as much as possible. Deoxyribonucleic acid (DNA) extraction utilized the kit provided by the company Geneaid (gSYNC^{TM} DNA Extraction Kit), following the steps according to the protocol of the company to extract the DNA from the tissue.

Polymerase chain reaction

The polymerase chain reaction (PCR) of the species pests infecting stored grains proceeded using the master mix prepared from Bioneer Company and a universal invertebrate primer with sequences as LCO1490 (5′ GGTC AACAAA TCATAAA GATATTGG -3′) and HCO2198 (5′ TAAACTTCAGGGTGACCAAAAAATCA -3′) to amplify portion of the mitochondria gene (mtCOXI-mitochondria Cytochrome Oxidase subunit I) (Folmer *et al*., 1994). This prepared primer came from the Macrogen Company as a dried product with different concentrations. Adding to each tube of the Master Mix 2 µl forward and reverse primers, 5 µl DNA of each species, and 6 µl nuclease free water before the tubes' placement in a ThermoCycle. The program used for amplification is as follows: initial denaturation, 5 min at 95 °C, denaturation, 1 min at 95 °C, annealing, 1 min at 49 °C, extension, 1 min at 72 °C, and final extension, 2 min at 27 °C, except for the *Rhyzopertha dominica* with the temperature annealing at 48 °C. The amplified gene for each species bore confirmation by electrophoresis on agarose gel with a concentration of 1%. The agarose gel's transfer to ultraviolet light helped examine the bands resulting from polymerase chain reactions. If bands appear, the camera can photograph them.

RESULTS AND DISCUSSION

Measurement of the DNA concentration

The extraction results showed the DNA concentration of five species of pests infecting stored grains. These are *Tribolium castaneum, Oryzaephilus mercator*, *Trogoderma granarium, Rhyzopertha dominica,* and *Callosobruchus maculatus* at 175, 171, 162, 182, and 193 ng/µL, respectively. Meanwhile, the purity was 1.81, 1.75, 1.96, 1.62, and 1.87, respectively.

Results of the polymerase chain reactions revealed the target gene appeared in the five species *Tribolium castaneum, Oryzaephilus mercator, Trogoderma granarium*, *Rhyzopertha dominica,* and *Callosobruchus maculatus,* at a molecular weight of 710 bp compared with the DNA ladder. Such studies can be beneficial in diagnosing species and conducting comparative studies between local species with those in the gene bank to know their occurring differences as a result of environmental conditions.

DNA sequences are one of the vital ways in diagnosing species and the extent of convergence or divergence between local species with those in different geographical locations (Pons *et al.,* 2006). The results' analysis of the PCR product continued in the Macrogen Company in Korea. After the alignment, the comparison of the DNA sequences of a partial of the mtCOXI gene of the five local species studied ensued with the sequences of those recorded in the NCBI GenBank in different parts of the world. The study used the BLAST search tool, which indicated existing mutations.

The sequences of partial of the mtCOXI gene of the five local species of pests infecting stored grains received accession numbers, as mentioned in Table 1. The percentage of match ranged from 95%–100% between the local species and the species registered in the GenBank (NCBI). The species *Tribolium*

Table 1. Accession number for the five local species and the accession number of related species in NCBT.

Species	Local species accession number in the GenBank	Accession of species that were close to local species in the GenBank
Tribolium castaneum	OR343196	OQ509919.1, OP604564.1, OP604557.1 ,KM439779.1, ON505589.1, ON482348.1
Oryzaephilus mercator	OR343197	MG458965.1
Trogoderma granarium	OR343199	LC386209.1 ,OQ358899.1, OP597472.1, HM398878.1
Rhyzopertha dominica	OR343198	MN271961.1, MH91005, KY440152.1, KM450240, OM882649.1, KC407717.1
Callosobruchus maculatus	OR343194	MG45897.1, MT641322.1, KU912434.1, MK649857.1, MT422564.1, MT42256.1, MT422540.1

castaneum was identical to the samples recorded in India, Germany, South Korea, and Mexico. However, the species *Oryzaephilus mercator* had the percentage closer between the local species and the species recorded in the USA at 0.003, with the Indian specimen at 0.006, and with Nigerian specimens at 0.008.

As for the species *Trogoderma granarium,* it is close to the Japanese samples and the samples in the USA, Australia, and Canada at the rate of 0.0016 and China at the rate of 0.0017. The species *Rhyzopertha dominica* is identical to the samples recorded in India, Germany, and South Korea. The species *Callosobruchus maculatus* is alike with the species recorded in the USA and differed from specimens recorded in India at 0.027.

Phylogenetic tree of the mtCOXI gene for five species of pests

The results of phylogenetic tree analysis of the five species of pests infecting stored grains in the promising study targeted the mtCOXI gene. The species *Tribolium castaneum* is genetically identical to species with accession numbers OQ509919.1, OP604564.1, and OP604557.1 from India, KM439779.1 from Germany, ON505589.1 from Mexico, and ON482348.1 from South Korea (Figure 1)**.**

The *Oryzaephilus mercator* is genetically close with species with accession numbers MG458965.1 from the USA, MH910051.1 from India, and MW728029.1 and MW728028.1 from Nigeria (Figure 2). The *Trogoderma granarium* is genetically similar to species with accession numbers LC386209.1 from Japan, OQ358899.1 from the USA, OP597472.1 from Australia, and HM398878.1 from Canada, with the same degree of convergence (Figure 3).

The species *Rhyzopertha dominica* is genetically identical to species with accession numbers MN271961.1, MH910056.1, KY440152.1, and OM882649.1 from India, KM450240.1 from Germany, and KC407717.1 from South Korea (Figure 4). The *Callosobruchus maculatus* is genetically identical with species with accession numbers MG458967.1 from the USA and close with species with accession number MT641322.1 from South Korea (Figure 5).

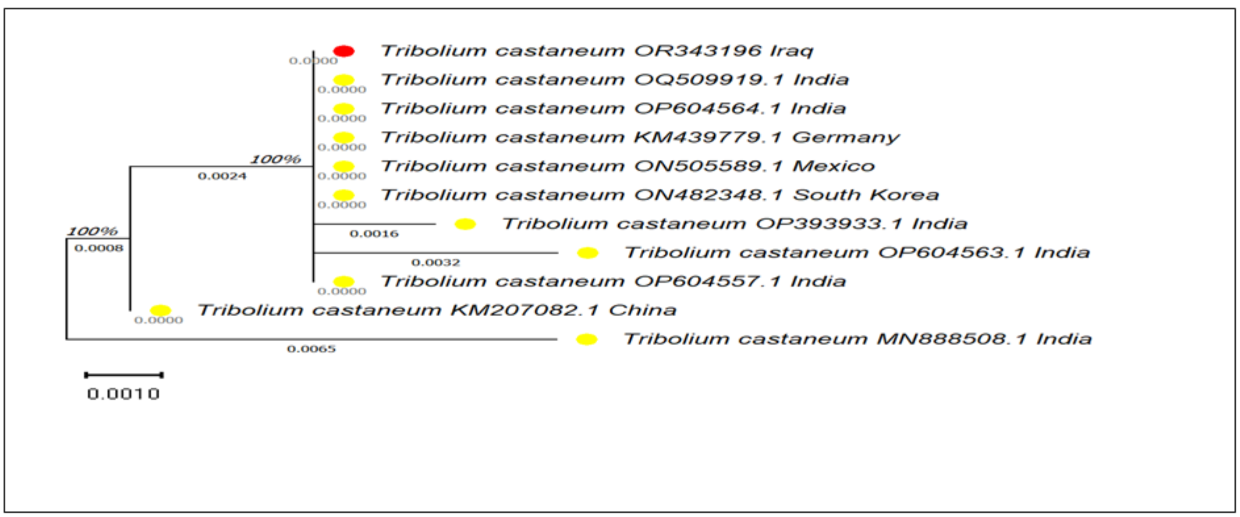

Figure 1. Phylogenetic tree of the mtCOXI gene of the pest*, Tribolium castaneum.*

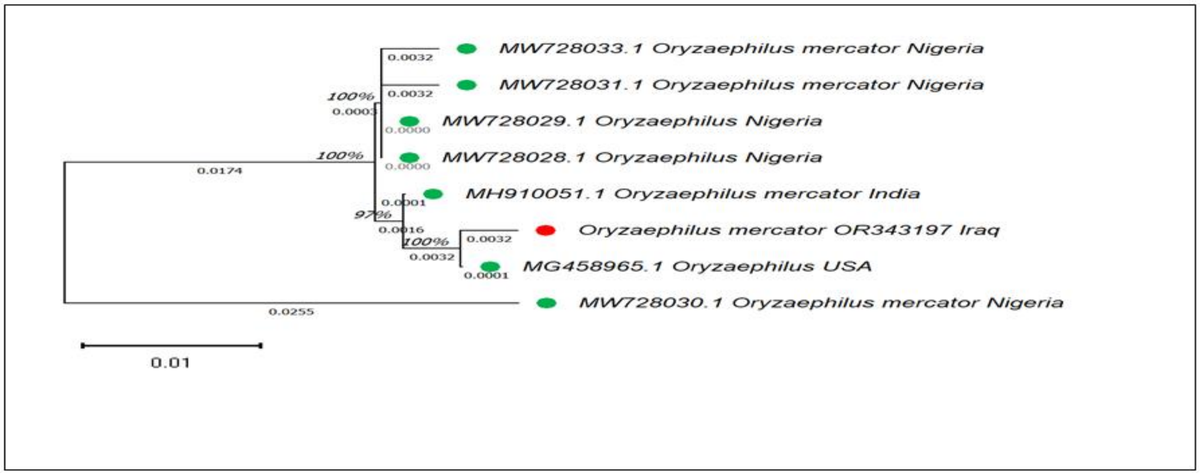

Figure 2. Phylogenetic tree of the mtCOXI gene of the pest, *Oryzaephilus mercator.*

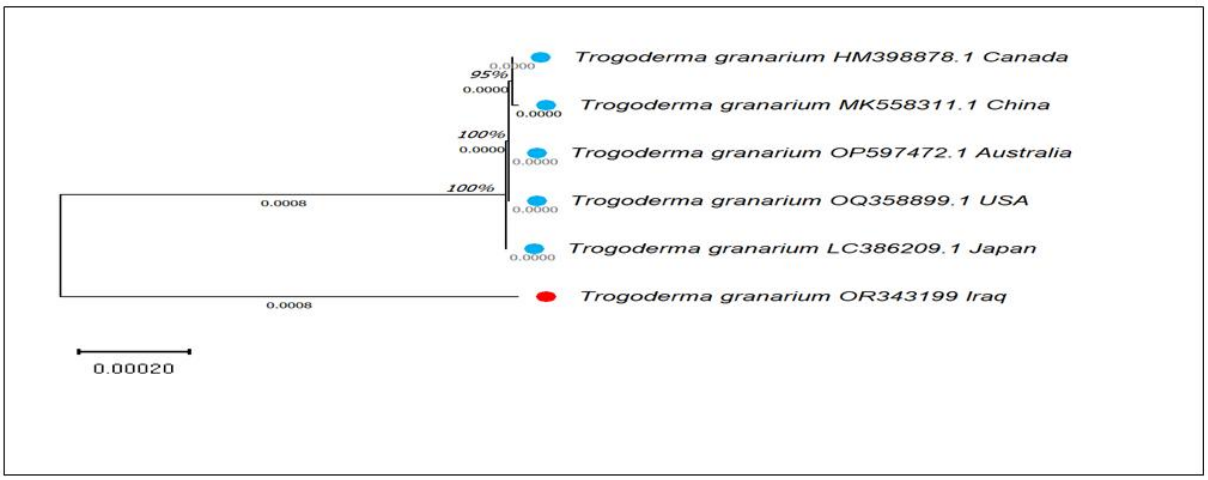

Figure 3. Phylogenetic tree of the mtCOXI gene of the pest, *Trogoderma granarium.*

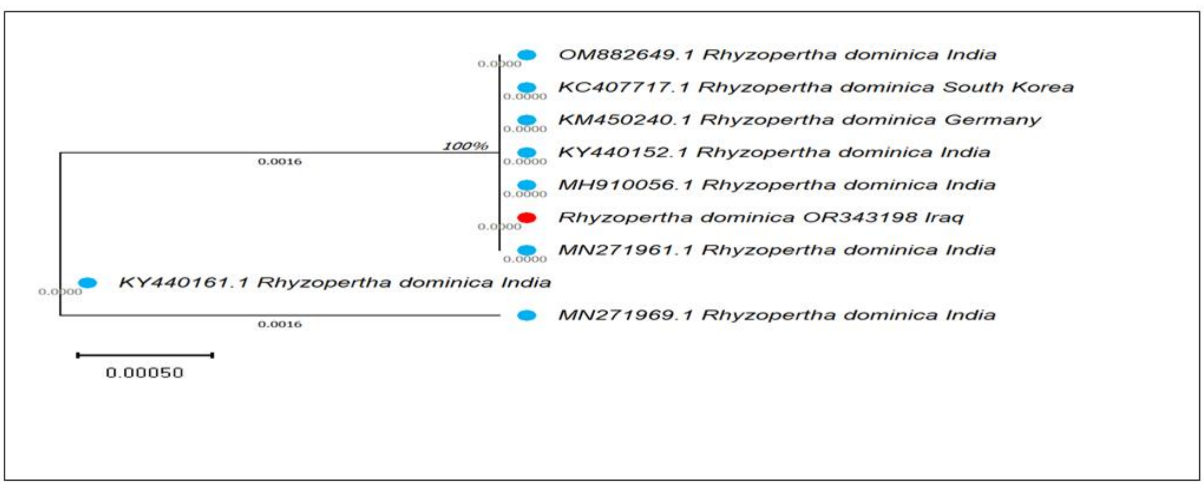

Figure 4. Phylogenetic tree of the mtCOXI gene of *Rhyzopertha dominica.*

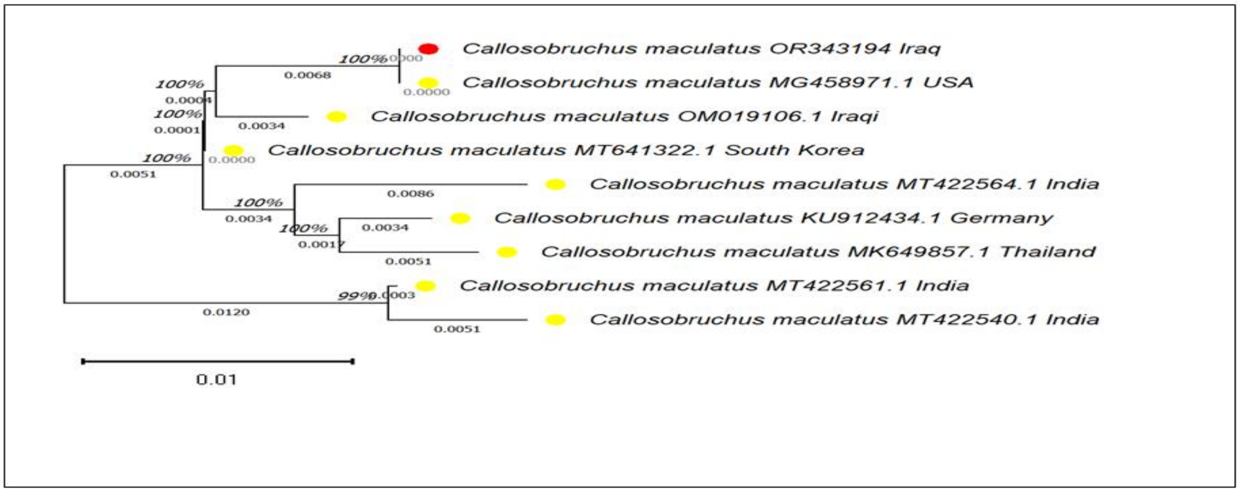

Figure 5. Phylogenetic tree of the mtCOXI gene of *Callosobruchus maculatus.*

Stored grains and their products are among the most essential main sources for humans and represent the chief part of food for many peoples worldwide. The presence of insects in these stored grains results in many damages, including secretions and undesirable odors, resulting from a substance called benzoquinones. This substance is one of the prime causes of cancerous diseases of various types and also causes allergic diseases in humans (Lu *et al*., 2012; Angadi *et al*., 2014).

More than 60 species of insects infect grains and their products, with most of these species belonging to the order Coleoptera and Lepidoptera (Jian, 2019; Abbasi *et al*., 2021). The species that retune to the order Coleoptera constitute the largest percentage of pests infecting grains, with among these 500 species found in grains and stored products, only 100 species feed on and damage grains and reduce their economic value. As for the remaining species, they feed on fungi contaminating grains and grow on them, while other pests feed on dead warehouse insects (Rajasri *et al*., 2020). Insects possess huge genetic diversity that can be detected using molecular techniques focusing on DNA markers. Differences in insects transcend beyond morphological characters. However, these manifest in behavior and size within an insect population and even within individuals of the same species (Petrson *et al*., 2012).

Detecting the biological diversity appearing in a species can proceed by analyzing a small piece of the organism's genome, using one or more genes for diagnosis. In this way, inexperienced researchers can obtain information in the field of identification and classification of unknown species and even the classification of new species. The genetic diversity available in pests of stored grains may correlate with the geographic origin of the strains infecting the grains, or there may be a problem with trade between countries (Ajayi and Rahman, 2006). Recently, DNA barcodes have served to confirm morphological identification. This method depends on the diversity that occurs in the sequences of the mtCOXI, known as the DNA barcode (Heberts *et al.,* 2003).

Studies using these markers have focused on molecular differences between and within the same species (Gupta and Jindal, 2013; Zufall *et al.*, 2013). The presented study diagnosed five species of pests infecting stored grains in Misan Province, Southern Iraq, using a universal primer and the PCR technique to amplify a part of a gene mtCOXI. It also obtained the gene’s sequence for five selected species for submission in the database of the GenBank (NCBI).

The rate of genetic match between local species and species registered in the GenBank as well as distributed in different parts of the world was from 95% to 100%. This included India, Germany, Mexico, and South Korea for the *Tribolium castaneum* species; the USA, India, and Nigeria for the *Oryzaephilus mercator* species; and Japan and the USA for the *Trogoderma granarium* species. Other species comprised India, Germany, and South Korea for the *Rhyzopertha dominica* species and the USA for *Callosobruchus maculatus*. This match ratio indicates the species of the pest maintained its genetic structure and moved with grains between continents, spreading and adapting to varying climate changes and different environments.

This study aimed to confirm the morphological diagnosis of the pests as recorded in stored grains in Misan Province, South Iraq. Many studies used the primer employed in the said study with different annealing temperatures to distinguish or diagnose different species in various parts of the world and even in different provinces of Iraq. Aslam *et al*. (2019a) distinguished two species similar in characters, making it difficult to distinguish between them. These two species are *Tribolium castaneum* and *Tribolium confusum,* with an annealing temperature 72 °C. Their research showed the two species with a molecular weight of 658 base pairs.

The same researcher also detected three species of pests infecting grains, and these species were *Callosobruchus chinensis, Sitophilus oryzae,* and *Oryzaephilus surinamensis* with a temperature annealing of 49 °C (Aslam *et al.,* 2019b). In China, identification of five species belonged to the genus *Cryptolestes,* as well as in Northern India and other geographical locations (Varadinova *et al*., 2015). Primer sequences to find out the genetic diversity occurring in the species *Rhyzopertha dominica* with an annealing temperature of 48 °C, and the bands appeared with a molecular weight of 700 base pairs, either in Iraq or elsewhere. Hamad and Sultan (2021) used the annealing temperature of 55 °C to diagnose the genetics of two species of *Tribolium castaneum* and *Tribolium confusum* from other studies in Iraq. Mzhr *et al.* (2022) made the molecular detection of pests infecting rice crops in Mosul, Baghdad, Saladin, Diyala, and Dhi Qar, using the same primer and showed bands belonging to the species after electrophoresis at a molecular weight of 700 bp.

Among the difficulties encountered during the presented study are the steps of extracting DNA, as Coleoptera needs more incubation periods when using adults compared with larvae or legs. It is due to the hard exoskeleton covering the entire body, which consists of chitin, complex proteins, and peptides, and the nature of these compounds. It reduces the efficiency of the materials used in the extraction and, thus, reduces the concentration and purity of the extracted DNA. For this reason, many modifications occurred in the methods of work. These modifications include an increase in incubation periods when using adults, from three hours to five hours or more. Continuous shaking ensued every 15 min for samples during the incubation period to ensure for the compounds to enter the tissue and destroy the tissue and cells to reach the DNA and extract it.

CONCLUSIONS

Polymerase chain reaction technology (PCR) is an effective and rapid means of diagnosing and confirming the analysis of species being difficult to diagnose morphologically or whose numbers are small.

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REFERENCES

Abbasi I, Halaseh L, Darwish HM, Matouk I (2021). Strategy for DNA extraction and detection from insect pests in stored home grain samples. *Al-Quds J. Acad. Res.* (1)1: 24–32.

Ajayi FA, Rahman SA (2006). Susceptibility of some staple processed meals to red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera:

Tenebrionidae). *Pak. J. Biol. Sci.* 9(9): 1744–1748.

Al-farhany ZL, Kareem DhK (2022). Molecular identification of five species of family Muscidae (insect: Diptera) from Basrah-South Iraq. *Ann. for Res.* 65(1): 3175–3184.

Angadi JG, Jahagirdar KA, Patil SL (2014). An extension strategy for promoting effective use of organic manures by farmers. *Int. J. Agric. Stat. Sci.* 10(1): 237–239.

Aslam AFM, Sultana S, Das SR, Howlader AJ (2019a). DNA barcoding to resolve the confusion in identifying *Tribolium confusum* and *Tribolium castaneum*. *Bangladesh J. Zool.* 47(2): 333–342.

Aslam AFM, Sultana S, Rain FF, Das SR, Siddika A, Howlader AJ (2019b). Molecular characterization and identification of three stored grain pests based on mitochondrial cytochrome c oxidase subunit I (COI) gene sequences. *Bangladesh J. Zool.* 47(1): 1–11.

Ball SL, Armstrong KF (2006). DNA barcodes for insect pest identification: A test case with tussock moths (Lepidoptera: Lymantriidae). *Can. J. For. Res.* 36(2): 337–350.

Ball SL, Hebert PDN, Burian SK, Webb JM (2005). Biological identification of May flies (Ephemenoptera) using DNA barcodes. *J.N.A.M. Benthol. Soc.* 24(3): 508–524.

Barrett RDH, Hebert PDN (2005). Identifying spiders through DNA barcodes. *Can. J. Zool.* 83(3): 481–491.

Folmer O, Black M, Hoeh W, Lule R, Vrijenhockt R (1994). DNA primers for amplification of mitochondrial cytochrome coxidase subunit I from diverse metazoan invertebrates. *Mol. Mari. Biol. Biol Technol.* 3(5): 294–299.

Gupta VK, Jindal V (2013). DNA barcoding: Universal platform for molecular taxonomy and identification*. Sci. Pub. (India) Jodhpur.* pp. 1–43.

Hamad SS, Sultan AA (2021). Molecular genetic diagnosis of *Tribolium castaneum* and *Tribolium confusum* (Coleoptera: Tenebrionidae) by sequencing of COI gene analysis. *Biochem. Cell Arch.* 21(2): 3619–3624.

Hebert PDN, Cywinska A, Ball SL, DeWaard JR (2003). Biological identifications through DNA barcodes. *Proc. Royal Soc. London. B Biol.* 270(1512): 313–321.

Jagshoran SR, Tripathi SC (2004). New varieties and production. *The Hindu. Surv. Indian Agric.* 4: 33–35.

Jian F (2019). Influences of stored product insect movements on integrated pest management decisions. *Insects* 10(4): 1–20.

Jinbo U, Kato T, Ito M (2011). Current progress in DNA barcoding and future implications for entomology. *Entomol. Sci.* 14(2): 107–124.

Kalaf JM (2021). Genetic diagnosis of the rust red flour beetle *Tribolium castaneum* (Herbst) (Coleoptera) and its mortality, attraction and repelling effect of some vegetable oil. *Int. J. Agric. State. Sci.* 17(1): 1867–1872.

Kumar D, Kalita P (2017). Reducing postharvest losses during storage of grain crops to strengthen food security in developing countries. *Foods* 6(1): 8.

Lu B, Lamora A, Sun Y, Welsh MJ, Ben-Shahar Y (2012). PPK 23-dependent chemosensory functions contribute to courtship behavior in *Drosophila melanogaster*. *Plos Genet.* 8(3): e1002587.

Mzhr NN, Jawad MM, Saber AJ (2022). Molecular detection of three coleopteran species found in stored rice from some provinces in Iraq*. Int. J. Health Sci.* 6(S3): 6756–6766.

Petrson BK, Weber JN, Kay EH, Fisher HS, Hoekstra HE (2012). Double digest RADseq: An inexpensive method for de novo SNP discovery and genotyping in model and non-model species. *PloS one.* 7(5): e37135.

Pimentel D (1991). World resources and food losses to pests. *Ecol. Manag. Food-indus*. Pests. 5–11.

Pons J, Barraclough T, Gomez-Zurita J, Cardoso A, Duran D, Hazell S, Kamoun S, Sumlin W, Vogler A (2006). Sequence-based species delimitation for the DNA taxonomy of undescribed insects*. Syst. Biol.* 55(4): 595–610.

Rajasri M, Sujeetha JARP, Gupta CS, Geetha SP (2020). Stored grain pests and their identification. National Institute of Plant Health Management https://www.researchgate.net/publication/341134532**.**

Rajendran S, Sriranjini V (2008). Plant products as fumigants for stored-product insect control. *J. Stor. Pro. Res.* 44(2): 126–135.

Rugman-Jones PF, Wharton VNRT, Stouthamer R (2009). Molecular identification of the *Psyttalia concolor* (Szépligeti species complex (Hymenoptera: Braconidae) associated with olive fly *Bactrocera oleae* (Rossi) (Diptera: Tephritidae) in Africa. *Biol. Control* 49(1): 17–26.

Timm AE, Warnich L, Geertsema H (2008). Morphological and molecular identification of economically important Toticidae (Lepidoptera) on tropical and subtropical fruit in South Africa. *Afr. Entomol.* 16(2): 209–219.

Timm AE, Warnichl L, Geertsema H (2007). Morphological and molecular identification of economically important Toticidae (Lepidoptera) on tropical and subtropical fruit in South Africa. *Afr. Entomol.* 15(2): 269–286.

Varadinova Z, Wang YJ, Kucerova Z, Stejskal V, Opit G, Cao Y, Li ZH (2015). COI barcode based species –specific – primers for identification of five species of stored product pests from genus *Cryptolestes* (Coleoptera: Laemophloeidae). *Bull. Entomol. Res.* 105(2): 202–209.

Zufall RA, Dimond KL, Doerder FP (2013). Restricted distribution and limited gene flow in the model ciliate *Tetrahymena thermophila*. *Mol. Ecol.* 22(4): 1081–1091.