RESEARCH PAPER

Molecular characterization and PCR detection of Aleuroglyphus ovatus infesting stored cattle feed

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ABSTRACT
Storage mites are common contaminants of stored agricultural commodities and livestock feed, posing risks to feed quality, animal health, and occupational safety. Among them, Aleuroglyphus ovatus is frequently reported from stored feed materials, yet its detection using conventional morphological methods can be difficult when mite populations are low or specimens are damaged. The present study aimed to develop a sensitive molecular assay for the detection of A. ovatus based on the internal transcribed spacer 2 (ITS2) region of ribosomal DNA. Genomic DNA isolated from A. ovatus was successfully amplified using ITS2 primers, generating fragments of approximately 460-480 bp. Sequencing of the amplified products yielded ITS2 sequences ranging from 455 to 465 bp. BLAST analysis confirmed the taxonomic identity of the samples as A. ovatus, showing 87-90% similarity with available reference sequences in the NCBI GenBank database. Based on the obtained sequence information, a species-specific primer pair targeting the ITS2 region was designed, producing a diagnostic amplicon of 180 bp. The developed PCR assay exhibited high analytical sensitivity and specificity, successfully detecting DNA extracted from single mite specimens. Sensitivity analysis demonstrated that the assay could detect A. ovatus DNA at 4000 pg/µL and detect a single mite in 100 mg of cattle feed. Application of the assay to 100 stored feed samples revealed A. ovatus DNA in 65% of the samples, indicating a high level of mite contamination in the surveyed feed materials. Overall, the ITS2-based PCR assay developed in this study provides a rapid, sensitive, and reliable tool for the molecular detection of A. ovatus in stored feed systems. The method may facilitate early detection and monitoring of storage mite infestations and support improved management strategies to maintain feed quality and safety.

Keywords: *Aleuroglyphus ovatus, storage mites, ITS2 rDNA, PCR detection, cattle feed.*
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INTRODUCTION

Stored cattle feed creates suitable conditions for the growth and multiplication of various arthropods. Among these, storage mites (Acari: Astigmata) are particularly important due to their frequent occurrence and association with feed deterioration. These mites infest cereal grains, feed concentrates, and fodder during storage, leading to quantitative losses, deterioration of nutritional quality, and contamination of feed with mite bodies, fecal matter, and allergens (Siegert *et al.,* 2018). Infestation of cattle feed by storage mites not only affects animal health and productivity but also poses occupational health risks to workers handling infested feed, including respiratory allergies, dermatitis, and asthma-like symptoms (Arcangeli *et al.,* 2020).

Among storage mites, *Aleuroglyphus ovatus* is widely distributed and frequently reported from stored agricultural commodities and animal feeds under warm and humid conditions. The species can rapidly grow and persist even in relatively dry feed substrates, making it a significant pest in feed storage systems. Accurate identification and early detection of *A. ovatus* infestation are therefore essential for implementing timely management strategies and preventing economic and health-related losses (Liao *et al.,* 2025).

Conventional identification of storage mites relies primarily on morphological examination under a microscope. Although morphological taxonomy remains the cornerstone of acarology, it requires well-preserved adult specimens, specialized expertise, and considerable time. Moreover, morphological identification becomes difficult when samples contain immature stages, damaged specimens, or low mite populations, as commonly encountered in early infestations (Kucerova and Stejskal

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2009). These limitations highlight the need for sensitive, rapid, and reliable molecular tools for the detection and identification of storage mites directly from feed matrices (Nain *et al.,* 2025).

Molecular diagnostic techniques based on polymerase chain reaction (PCR) have emerged as powerful alternatives to traditional methods for mite identification. Ribosomal DNA (rDNA) regions, particularly the internal transcribed spacer 2 (ITS2), have been extensively used for species-level identification in mites due to their high interspecific variability and conserved flanking regions (Wong *et al.,* 2011). ITS2-based PCR assays offer high sensitivity and specificity and can detect target species even from minute quantities of DNA, making them suitable for samples with low mite density or mixed biological material such as animal feed (Mushtaq *et al.,* 2023).

Despite the increasing use of molecular tools in mite taxonomy and diagnostics, limited information is available on PCR-based detection of *A. ovatus* in stored cattle feed, especially under Indian agroclimatic conditions (Verma 2023). Furthermore, the comparative evaluation of DNA extraction methods and PCR detection efficiency in both individual mites and mite-infested feed samples remains underexplored. Developing a laboratory-validated molecular detection approach is therefore essential for reliable identification and monitoring of *A. ovatus* infestation in cattle feed storage systems.

The present study was undertaken to develop and validate a PCR-based molecular detection method for *Aleuroglyphus ovatus* using the ITS2 region of rDNA. Genomic DNA was extracted from different numbers of mites and mite-infested cattle feed samples using a modified CTAB protocol and a commercial spin-column kit, followed by assessment of DNA quality and quantity. PCR amplification of the ITS2 region, sequencing, and sequence homology analysis were performed to confirm species identity. The developed PCR assay was further validated using laboratory-cultured mites and field-collected feed samples to evaluate its applicability for the routine detection of *A. ovatus* infestation.

This study provides a sensitive and reliable molecular approach for detecting *Aleuroglyphus ovatus* in stored cattle feed and enhances diagnostic capability for monitoring storage mite infestations. The findings are expected to support improved feed hygiene management and reduce the risks of mite contamination in cattle feed storage systems.

MATERIALS AND METHODS

1. Collection and laboratory culture of mites

During the survey of cattle feed samples, *Aleuroglyphus ovatus* was found to be the predominant storage mite species. Live mites were extracted from infested cattle feed samples under a stereomicroscope using a fine hair brush. Extracted mites were cultured under lab condition as a stock culture for molecular analysis. For culturing, 10 extracted adult mites were released in plastic petri dishes (5 cm diameter) containing 5g of cattle feed concentrate. A small hole was made in the lid and covered with muslin cloth to allow aeration while preventing mite escape. The Petri dishes were placed in desiccators containing a supersaturated potassium chloride solution to maintain relative humidity and incubated in a BOD incubator at 27±1°C (Xia *et al.,* 2012). Cultured mites were used for DNA extraction and PCR standardization.

2. Extraction of genomic DNA

Genomic DNA was extracted from individual mites and from mite-infested cattle feed samples (Table 2) using two methods to evaluate extraction efficiency.

2.1.1 DNA extraction using the modified CTAB method

A modified CTAB-based protocol was followed for genomic DNA extraction from *A. ovatus* mites. Different numbers of mites (1, 5, 10, 15, 20, and 30) were picked from the stock culture, washed with 70% ethanol, and transferred into 1.5 ml microcentrifuge tubes. Proteinase K (2 µl, 20 mg/ml) and 100 µl CTAB extraction buffer were added, and the samples were homogenized using a plastic micropipette tip. The homogenates were incubated at 65°C for 1 h to facilitate cell lysis. After incubation, 100 µL of chloroform: isoamyl alcohol (24:1) was added, and the mixture was gently mixed. Samples were centrifuged at 12,000 rpm for 15 min, and the aqueous phase was transferred to a fresh tube. DNA was precipitated by adding ice-cold isopropanol and incubating at -20 °C for 2 h. The DNA pellet was recovered by centrifugation, washed with 70% ethanol, air-dried, and finally resuspended in 20 µL of TE buffer, then stored at -20°C (Yousefnejad *et al.,* 2026).

2.2 DNA extraction from mite-infested feed samples

For the extraction of DNA from mite-infested feed samples, 100 mg of sieved feed was crushed in CTAB buffer using a sterile mortar and pestle. Proteinase K was added, and the samples were incubated at 65°C for 1 hour. Chloroform: isoamyl alcohol extraction, DNA precipitation, washing, and elution were performed using the same protocol as for mite DNA extraction. DNA was finally eluted in 100 µl TE buffer and stored at -20 °C (Ta-Phaisach *et al.,* 2023).

2.3 DNA extraction using DNase Blood & Tissue Kit

Genomic DNA from different numbers of mites and mite-infested feed samples was also extracted using the DNase Blood & Tissue Kit (Qiagen) following the manufacturer's spin-column protocol. DNA was eluted in AE buffer and stored at -20 °C until further use (Jiang *et al.,* 2026).

3. Quality and quantity assessment of genomic DNA

The quality and quantity of extracted DNA were assessed using a Nanodrop UV spectrophotometer. DNA concentration was measured at 260 nm, and purity was assessed by calculating the A_{260}/A_{280} ratio (Gutiérrez-Contreras *et al.,* 2026). DNA samples showing A_{260}/A_{280} values between 1.8 and 2.0 were considered suitable for

PCR amplification. Each sample was measured in triplicate.

4. PCR amplification of the ITS2 region

PCR amplification of the ITS2 region of ribosomal DNA was performed using ITS2 forward and reverse primers (Table 1) specific for astigmatic mites. PCR reactions were carried out in a 25 µl reaction volume containing 2X PCR master mix, forward and reverse primers (10 µM each), template DNA (5-50 ng), and nuclease-free water. Amplification was performed in a thermal cycler with an initial denaturation at 94°C for 4 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 1 min, with a final extension at 72°C for 7 min. PCR products were stored at -20°C until further analysis (Xin *et al.,* 2018).

Table 1: ITS2 forward and reverse primers used for PCR amplification for astigmatid mites.

S.No.	Locus	Primer	Sequence	No. of Bases
1	ITS2 F 5'-3'	Forward(F)	CGACTTTCGAACGCATATTGC	21
2	ITS2 R 5'-3'	Reverse(R)	GCTTAAATTCAGGGGGTAATCTCG	24

5. Agarose gel electrophoresis

PCR products were resolved by agarose gel electrophoresis using a 1.5% agarose gel prepared in 1X TAE buffer. PCR products mixed with loading dye were loaded alongside a 100 bp DNA ladder. Electrophoresis was carried out at 65 V until adequate separation was achieved (Liu *et al.,* 2025). Amplified DNA fragments were visualized under a gel documentation system.

6. PCR product sequencing

Selected PCR products (M1, M2, S1, and S2) obtained from mite DNA and mite-infested feed samples were purified and subjected to DNA sequencing through a commercial sequencing facility. Both forward and reverse primers were used to sequence the ITS2 region (Sun *et al.,* 2014).

7. Sequence analysis

The obtained ITS2 sequences were edited and assembled into consensus sequences using BioEdit software. Species identity was confirmed by comparing the consensus sequences with available sequences in the GenBank database using the BLASTN tool (Sun *et al.,* 2014).

8. Primer designing:

The ITS2 amplicon sequences from samples (M, M2, S1, and S2) were aligned using ClustalW to identify the most appropriate regions for primer design, and primers were designed with Primer3web version 4.1.0. Primers were designed using the parameters: 18-25 bp length (optimal 20 bp), melting temperature (Tm) of 55-60°C, and GC content up to 60%. The primer set retrieved was analyzed with Oligo Analyzer (JustBio) to validate primer parameters (Xu *et al.,* 2018).

9. In-silico analysis of the specificity of the newly designed primer and PCR amplification

To verify the specificity of the newly designed primer sets for the target species of storage mite, 50 astigmatid mite DNA sequences of the internal transcribed spacers (ITS) 2 present in GenBank, representing both house dust mite species and storage mite species, were retrieved in FASTA form (Kalendar *et al.,* 2024). These retrieved sequences were used, in order to perform 'in silico' primer hybridization analysis using FastPCR software. Specifically, Lepidoglyphus destructor, Tyrophagus putrescentiae, T. longior, Acarus siro, Rhizoglyphus robini, Suidasia nesbitii, S. medanesis, Sancassaina sp. (storage mite species), and *Dermatophagoides pteronyssinus, D. farinae, D. microceras, Blomia tropicalis* (house dust mite) were included.

10. Validation of PCR-based detection

The validated PCR assay was applied to genomic DNA extracted from laboratory-cultured mites and mite-infested cattle feed samples to confirm the presence of *Aleuroglyphus ovatus*. Detection sensitivity was evaluated using different numbers of mites and varying infestation levels in feed samples. Presence or absence of amplification was determined based on visualization of specific DNA bands on agarose gel electrophoresis.

RESULTS

1. Genomic DNA quality and yield

Genomic DNA obtained from *Aleuroglyphus ovatus* samples showed considerable variation in yield depending on the number of mites used for extraction (Table 2). DNA concentration increased progressively with increasing sample size, indicating efficient recovery of nucleic acids from mite tissues. The highest DNA concentration **(108 ng/µL) was observed in sample S7, which contained** 50 mites. Samples containing intermediate numbers of mites produced proportionally lower DNA concentrations, whereas extraction from a single mite yielded approximately **4.9 ng/µl** DNA. Spectrophotometric analysis revealed that the extracted DNA exhibited **A_{260}/A_{280} ratios of 1.70-2.00**, indicating acceptable purity with minimal protein contamination. DNA samples extracted with the commercial kit generally exhibited slightly higher purity values than CTAB-extracted samples, although the CTAB method yielded higher DNA concentrations. The NanoDrop spectrophotometric profiles further confirmed the presence of intact nucleic acids in the extracted samples. These observations indicate that sufficient quantities of high-quality genomic DNA were obtained from *A. ovatus* samples for subsequent PCR amplification and molecular analysis.

Table 2: Quantity of DNA extracted from mites only and mites with feed.

S.No.	Code	Source of DNA extraction	Extraction by modified CTAB		Extraction by DNA extraction kit	
			Quantity(ng/µl)	A260/A280	Quantity(ng/µl)	A260/A280

1	M1	Only 1 mite	6.30	1.83	4.90	1.91
2	M2	5 mites	11.50	1.88	10.60	1.88
3	M3	10 mites	19.20	1.79	17.70	1.94
4	M4	15 mites	25.70	1.77	23.10	1.83
5	M5	20 mites	38.30	1.79	35.00	1.90
6	M6	30 mites	62.20	1.71	57.40	1.88
7	S1	Stock culture having 1 mite	42.90	2.03	31.70	1.99
8	S2	Stock culture having 5 mites	48.40	2.09	36.30	1.92
9	S3	Stock culture having 10 mites	53.10	2..01	40.20	1.88
10	S4	Stock culture having 15 mites	56.70	2.04	46.70	1.81
11	S5	Stock culture having 20 mites	65.30	1.97	53.00	1.93
12	S6	Stock culture having 30 mites	77.20	2.00	62.90	1.94
13	S7	Stock culture having 50 mites	108.00	2.10	88.30	2.00

2. Amplification of the ITS2 region

PCR amplification targeting the ITS2 region produced a distinct DNA fragment of approximately **460-480 bp**, corresponding to the expected ITS2 amplicon size for *A. ovatus*. Agarose gel electrophoresis revealed a clear single band in all successfully amplified samples (Figures 1 and 2), indicating specific amplification of the target locus. Amplification was consistently observed across samples with varying DNA concentrations, including DNA extracted from a single mite, demonstrating the assay's sensitivity. The amplified products were clearly resolved on agarose gels without the appearance of secondary bands or primer-dimer formations.

Additional electrophoretic analysis confirmed the reproducibility of amplification across multiple samples. The presence of distinct bands of similar size in all amplified samples indicates that the ITS2 region can serve as a reliable molecular marker for the identification of *A. ovatus*.

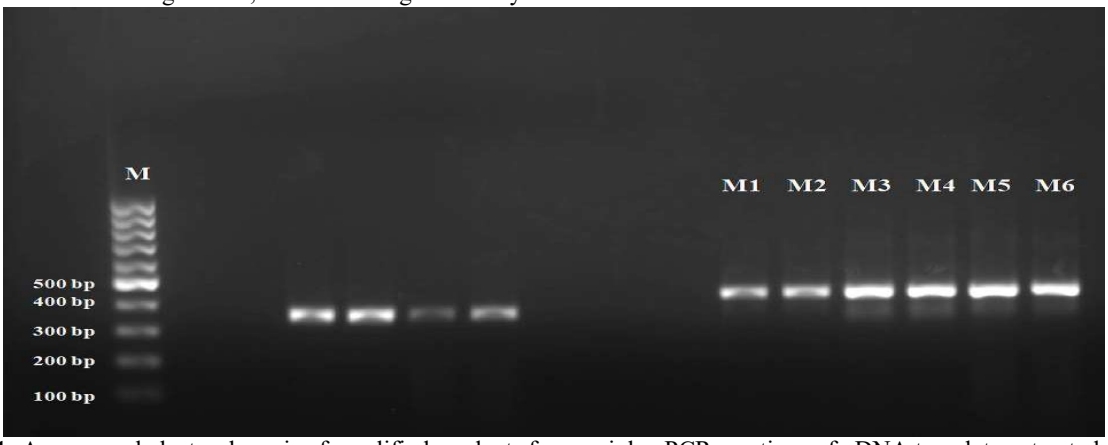

Fig 1: Agarose gel electrophoresis of amplified products from uniplex PCR reactions of gDNA template extracted from A. ovatus (varying no. of mites) with a varied amount of gDNA template

M-100 bp DNA ladder, Promega, USA; M1-amplicon of gDNA extracted from a single mite, M2, M3, M4, M5, and M6 amplicon of gDNA extracted from 5, 10, 15, 20, and 30 mites.

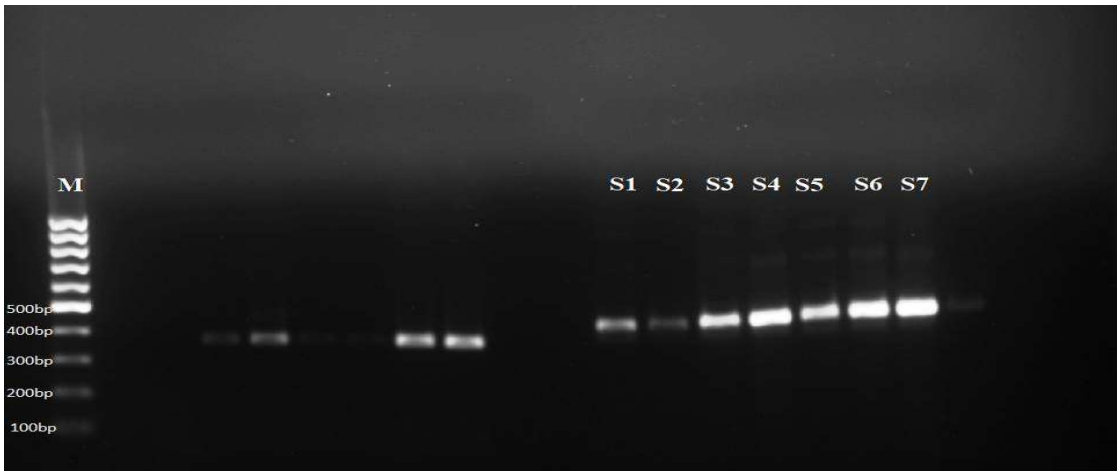

Fig 2: Agarose gel electrophoresis of amplified products from uniplex PCR reactions of gDNA template extracted from stock culture of *A. ovatus* (varying no. of mites in 100 mg of stock culture) with a varied amount of gDNA template

M-100 bp DNA ladder, Promega, USA; S1- amplicon of gDNA extracted from 100 mg stock culture having a single mite, S2, S3, S4, S5, S6, and S7 amplicon of gDNA extracted from 100 mg stock culture with 5, 10, 15, 20, 30, and 50 mites.

3. ITS2 sequence analysis

Sequencing of the amplified ITS2 fragments yielded high-quality sequences ranging from 455 to 465 bp (Table 3). BLAST analysis of the obtained sequences against the NCBI GenBank database confirmed their identity as *Aleuroglyphus ovatus*. The sequences showed **87-90% similarity** with previously reported ITS2 sequences of *A. ovatus* available in the GenBank database. The closest matches corresponded to sequences reported from **China and Japan**. Alignment analysis indicated that the obtained sequences clustered with previously reported *A. ovatus* sequences, confirming the species identity of the mite samples analyzed in the present study. Although minor sequence variation was observed in comparison with previously reported sequences, this variation may reflect **geographical genetic diversity among** *A. ovatus* **populations** or the limited availability of ITS2 reference sequences in public databases.

Table 3: Analysis of sequences by BLASTN of four samples of storage mite

Sequence ID	Locus	Base pairs	Identity (%)	E-value	Species	GenBank accession no.
M1 ITS2	ITS2	464	88	4e-39	*Aleuroglyphus ovatus*	KJ872604
M2 ITS2	ITS2	462	88	2e-37	*Aleuroglyphus ovatus*	AB104986
S1 ITS2	ITS2	460	87	2e-36	*Aleuroglyphus ovatus*	GQ205584
S2 ITS2	ITS2	455	90	8e-41	*Aleuroglyphus ovatus*	KJ872604

4. Species-specific primer validation

Based on the ITS2 sequence data, a species-specific primer pair was designed for rapid molecular detection of *A. ovatus*. PCR amplification using this primer set produced a distinct **180 bp fragment**, corresponding to the expected diagnostic amplicon size (Table 4). Electrophoretic analysis confirmed that the primers generated a single specific amplification product without the appearance of additional bands (Figure 3). Amplification was successfully obtained from DNA samples derived from the target species, indicating high primer specificity. The primer set also demonstrated high analytical sensitivity, as amplification was observed even with DNA extracted from single mite samples as the template.

Table 4: Species-specific *Aleuroglyphus ovatus* forward (Fwd) and reverse (Rev) primers and their analytical characteristics

Species	Target	Expected Amplicon length(bp)	Primer	Sequence (5'-3')	Length (bp)	GC (%) content	Tm (°C)
Aleuroglyphus ovatus	ITS2	180	Alu-Fwd	GCCTGTTCATCGTGTCAGTC	20	55	54
			Alu-Rev	GCTCAGGTGCTAATGACGTG	20	55	54

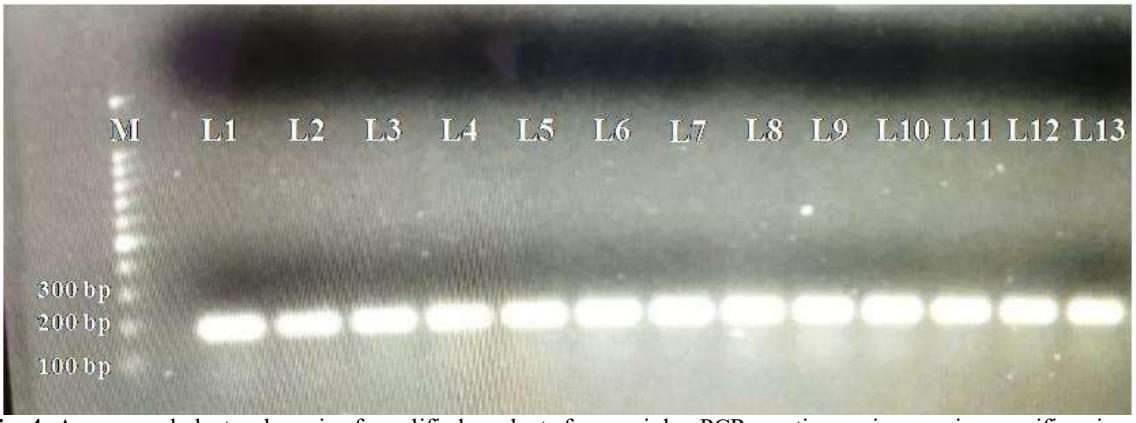

Fig. 4: Agarose gel electrophoresis of amplified products from uniplex PCR reactions using species-specific primers (Alu-Fwd and Alu-Rev) of gDNA template extracted from *A. ovatus* (varying number of mites) and stock culture of mite with different mite no with varied amounts of gDNA template.

M-100 bp molecular size marker (DNA ladder, Promega, USA); L1-L6-amplicon of gDNA extracted from single mite to 30 mites, while L7-L13 amplicon of gDNA extracted from stock culture of mites with one mite,5, 10, 15, 20, 30, and 50 mites.

5. Detection sensitivity of the PCR assay

Serial dilution experiments demonstrated that the PCR assay could detect *A. ovatus* DNA at concentrations as low as 4000 pg/µL. Amplification of the diagnostic **180 bp fragment** was consistently observed at this concentration, indicating the assay's high sensitivity. In addition, the assay successfully detected mite DNA when **a single mite was present in 100 mg of cattle feed**, confirming the ability of the developed PCR method to detect very low levels of infestation in feed samples.

6. PCR detection of *A. ovatus* in feed samples

Application of the species-specific PCR assay to **100 cattle feed samples** collected from different storage locations revealed the presence of *A. ovatus* DNA in **65 samples**, while **35 samples showed no detectable amplification**. Positive samples produced the expected **180 bp amplification product,** confirming infestation by the target mite species. In several samples with extremely low mite numbers, PCR amplification still produced a positive result, demonstrating higher sensitivity than microscopic examination. The agreement between microscopic observation and PCR detection further supports the reliability of the developed molecular assay for identifying *A. ovatus* in stored cattle feed samples.

DISCUSSION

Accurate detection and identification of storage mites are essential for effective monitoring of feed contamination and for minimizing economic and health-related risks associated with mite infestation (Sárkány *et al.,* 2025). In the present study, a PCR-based molecular detection assay targeting the ITS2 region of ribosomal DNA was successfully developed and validated for the identification of *Aleuroglyphus ovatus*. The results demonstrated that the developed assay has high sensitivity, specificity, and reproducibility for detecting *A. ovatus* in both pure mite samples and cattle feed matrices.

The quantity and quality of genomic DNA obtained in the present investigation varied with the number of mites used for extraction. DNA concentration increased progressively with increasing mite number, indicating efficient recovery of nucleic acids from mite tissues. Similar observations have been reported in previous molecular studies of astigmatid mites, in which DNA yield was positively associated with sample biomass and mite density (Zhao *et al.,* 2016). The modified CTAB method yielded higher DNA concentrations than the commercial extraction kit, whereas the kit-based extraction produced DNA with higher purity, as reflected in A_{260}/A_{280} ratios closer to the optimal range of 1.8-2.0. These findings are consistent with earlier reports demonstrating that CTAB extraction efficiently recovers nucleic acids from arthropod tissues but may co-extract secondary contaminants such as proteins or polysaccharides (Behura and Kothakota 2026). Nevertheless, the extracted DNA quality obtained in the present study was sufficient for downstream PCR amplification and sequencing analysis.

Amplification of the ITS2 region consistently generated a distinct fragment of approximately 460-480 bp from all tested samples, including DNA extracted from a single mite specimen. The absence of non-specific amplification and primer-dimer formation indicated high specificity of the selected ITS2 primers. Ribosomal ITS regions, particularly ITS2, are widely used as molecular markers for species-level identification because they contain conserved flanking regions along with species-specific sequence variability. Previous studies on storage mites and other acarid mites have also reported successful utilization of ITS2 sequences for molecular taxonomy and diagnostic assay development (Shamim *et al.,* 2017). The reproducibility of amplification across samples with varying DNA concentrations further demonstrates the robustness of the developed PCR conditions.

Sequence analysis of the amplified ITS2 fragments confirmed the identity of the analyzed mite samples as *A.*

ovatus. The obtained sequences exhibited 87-90% similarity with reference sequences available in the GenBank database. Although sequence identity values were slightly lower than those commonly reported for highly conserved loci, similar levels of variation have been observed in geographically distinct populations of arthropods and mites (Noge *et al.,* 2005). The observed sequence divergence may therefore reflect intraspecific genetic variability among *A. ovatus* populations originating from different geographical regions. Another possible explanation is the limited representation of *A. ovatus* ITS2 sequences in public nucleotide databases. Despite minor sequence variation, BLAST analysis consistently identified the obtained sequences as *A. ovatus*, thereby validating the taxonomic identity of the analyzed specimens (De Hoog and Horré 2002).

Based on the generated ITS2 sequence information, a species-specific primer pair was successfully designed for rapid molecular detection of *A. ovatus*. The designed primer pair amplified a single diagnostic fragment of 180 bp without generating secondary amplification products, indicating excellent primer specificity. Species-specific primers targeting ITS regions have been widely used for detecting mites, insects, fungi, and other microorganisms, as spacer regions exhibit high interspecific variability. In the present study, amplification was consistently achieved from DNA isolated from individual mites and from mite-contaminated feed samples, confirming the assay's applicability for low-template DNA detection.

Sensitivity analysis revealed that the developed PCR assay could detect genomic DNA concentrations as low as 4000 pg/µL and successfully identify the presence of a single mite in 100 mg of cattle feed. The ability to detect extremely low infestation levels is particularly important in stored feed systems because early stages of mite infestation often remain undetectable through conventional microscopic examination. Earlier studies on storage mite diagnostics have similarly demonstrated that PCR-based approaches have substantially higher analytical sensitivity than morphological detection methods (Roussel *et al.,* 2013). The high detection sensitivity observed in the present investigation may be attributed to the specificity of the ITS2-targeted primers and the optimization of PCR conditions.

Application of the developed PCR assay to field-collected cattle feed samples further demonstrated its practical utility for routine screening of storage mite infestation. Out of 100 analyzed feed samples, 65 samples tested positive for *A. ovatus*. Positive amplification was obtained even in samples containing very low mite populations, indicating that molecular detection could identify infestations that might otherwise be overlooked during microscopic examination. The high agreement between PCR results and microscopic observations confirms the reliability of the developed assay for detecting *A. ovatus* in stored feed materials. Moreover, the assay's ability to detect mites directly from feed matrices demonstrates its potential for surveillance programs, feed quality assessment, and quarantine inspections.

Overall, the present study establishes the ITS2 region as an effective molecular target for the identification of *A. ovatus* and demonstrates the usefulness of species-specific PCR as a rapid and reliable diagnostic tool for storage mite detection. The developed assay may facilitate early detection of infestations in stored feed systems, thereby supporting timely management interventions and reducing losses associated with mite contamination.

CONCLUSION

The present study successfully developed and validated a species-specific PCR assay targeting the ITS2 region of ribosomal DNA for the rapid detection and identification of *Aleuroglyphus ovatus*. The assay demonstrated high specificity, sensitivity, and reproducibility, enabling reliable detection of mites in individual mite specimens and in mite-contaminated cattle feed samples. DNA extracted using both CTAB and commercial kit-based methods was suitable for PCR amplification, while sequence analysis confirmed the taxonomic identity of the analyzed specimens. The designed species-specific primers produced a distinct 180bp diagnostic amplicon without non-specific amplification and were capable of detecting low levels of infestation, including a single mite in 100 mg of cattle feed. Application of the assay to field samples further demonstrated its effectiveness for routine monitoring of storage mite contamination. Overall, the developed ITS2-based molecular diagnostic tool provides a rapid, accurate, and practical approach for the early detection of *A. ovatus*, supporting improved feed quality management and helping to minimize the economic and health risks associated with storage mite infestations.

Conflict of interests

The authors declare that there is no conflict of interest.

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