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Short Article

# Comparative evaluation of DNA extraction methods from smut fungi teliospores

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## ABSTRACT

Smut fungi, particularly those within the genera *Tilletia*, *Ustilago*, *Sporisorium*, and *Urocystis*, threaten cereal crops worldwide. Accurate molecular identification of these pathogens requires high-quality genomic DNA, however, extracting DNA from smut teliospores remains a challenge due to their thick and resilient cell walls. To address this, four commonly used DNA extraction methods — Murray & Thompson, Raeder & Broda, Chelex 100 and HotSHOT — were comparatively evaluated across ten species of smut fungi: *Tilletia laevis*, *T. caries*, *T. controversa*, *T. indica*, *Ustilago tritici*, *U. nuda*, *U. hordei*, *Sporisorium maydis*, *S. ehrenbergii*, and *Urocystis agropyri*. Mechanical disruption using sterilized carborundum, with or without liquid nitrogen, was applied uniformly across all methods. The quality and quantity of extracted DNA were assessed by Nanodrop spectrophotometry and PCR amplification using ITS1/ITS4 primers. All methods yielded PCR-amplifiable DNA; however, substantial differences were observed in yield and purity. Raeder & Broda method yielded the highest average DNA concentration (1050.55 ng/µl), followed by HotSHOT (951.33 ng/µl), Murray & Thompson (918.82 ng/µl), and Chelex (879.37 ng/µl). While HotSHOT offered a higher yield than Murray & Thompson and Chelex, its lower purity suggested co-purification of cellular contaminants. Murray & Thompson and Raeder and Broda-extracted DNA exhibited better overall purity based on their average A260/A280 and A260/A230 ratios. Electrophoresis of PCR products revealed strong and consistent bands for all samples, indicating successful DNA amplification. This study demonstrates that Murray & Thompson remains optimal when purity is crucial, while Raeder & Broda is effective for higher-yield applications. HotSHOT and Chelex, despite lower purity, remain useful for rapid and routine diagnostics.

## KEYWORDS

Bunts and smuts, Chelex, HotSHOT, Thick-walled spore



## INTRODUCTION

Smut fungi represent an important group of plant pathogens within the phylum *Basidiomycota*, subphylum *Ustilaginomycotina*, and class *Exobasidiomycetes*. These pathogens are primarily associated with cereal crops, where they cause significant yield losses and compromise grain quality and international trade. Members of the genera *Tilletia*, *Ustilago*, *Urocystis*, and *Sporisorium* infect a wide range of grasses, including major staples such as wheat, barley, and maize. Among these, bunt diseases caused by *Tilletia* species, such as *T. laevis* and *T. caries* (causal agents of common bunt) and *T. controversa* (causal agent of dwarf bunt), are of major phytosanitary concern due to the mass production of teliospores that are difficult to eradicate and can persist in soil for several years (Grey et al. 1986, Goates 1996).

Teliospores are the primary dispersal and survival structures of smut fungi. They are thick-walled, heavily melanized, and highly resistant to environmental stress and degradation. These properties, while beneficial for fungal survival, present a major obstacle in the

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molecular detection and characterization of smut pathogens. The resistant nature of the teliospore wall, composed of chitin, glucans, and melanin, complicates cell lysis and often results in low DNA yield and purity, which can inhibit downstream molecular applications such as polymerase chain reaction (PCR) and loop-mediated isothermal amplification (LAMP) (Durán and Fischer 1961, Fernandez and Durán 1978, Notomi et al. 2000).

Accurate and rapid identification of smut pathogens at the species level is crucial for disease surveillance, breeding for resistance, epidemiological studies, and regulatory decision-making. Traditional diagnostic methods relying on morphology and symptomatology are often insufficient due to the similarity among species and the latent nature of infections. Molecular diagnostics, particularly DNA-based techniques such as PCR and LAMP, have emerged as reliable alternatives due to their high specificity, sensitivity, and rapid turnaround time (Murray and Thompson 1980, Notomi et al. 2000, Fuentes-Dávila et al. 2002). However, the effectiveness of these tools depends fundamentally on the ability to extract high-quality, inhibitor-free DNA from challenging fungal structures such as teliospores.

Several DNA extraction protocols have been developed and modified to address the limitations posed by fungal spores and other resistant structures. The Murray and Thompson method also known as cetyltrimethylammonium bromide (CTAB) (Murray and Thompson 1980), originally designed for plant DNA extraction, uses strong detergents and organic solvents (e.g., chloroform-isoamyl alcohol) to remove polysaccharides and phenolic compounds that can co-purify with DNA and inhibit enzymatic reactions. The Raeder & Broda method (Raeder and Broda 1985), designed specifically for filamentous fungi, is a rapid alkali-based protocol that uses sodium hydroxide and mechanical disruption with glass beads to facilitate cell wall breakdown. It offers the advantage of speed and simplicity, particularly for labs with limited resources. The Chelex 100 protocol (Walsh et al. 1991) employs a resin that binds divalent metal ions, thereby inactivating DNases and protecting DNA from degradation. This method is fast, requires minimal equipment, and is particularly useful for PCR-based diagnostics. The Hot Sodium Hydroxide and Tris (HotSHOT) method (Truett et al. 2000) is another alkaline lysis protocol, widely used in mammalian and microbial DNA extraction, which involves heating cells in NaOH followed by neutralization with Tris-HCl buffer. It is known for its speed and suitability for high-throughput applications.

Despite the wide use of these protocols in various biological contexts, there has been no systematic, comparative study focused on DNA extraction from smut fungi teliospores. Given the unique structural features of these spores, it is critical to assess the effectiveness of each method in terms of DNA yield, purity, and amplifiability, particularly when targeting species involved in regulated diseases such as dwarf bunt.

This study aims to address this gap by evaluating four widely used DNA extraction methods— Murray & Thompson, Raeder & Broda, Chelex 100, and HotSHOT—across teliospores from ten smut species: *Tilletia laevis*, *T. caries*, *T. controversa*, *T. indica, Ustilago tritici*, *U. nuda*, *U. hordei*, *Urocystis agropyri*, *Sporisorium maydis* and *S. ehrenbergii*. Each method will be assessed based on total DNA yield, purity ratios (A260/280), time and labor requirements, and the success of PCR amplification. By providing a clear, evidence-based comparison of extraction performance, this work will inform best practices for smut DNA diagnostics and support broader efforts in plant pathology, quarantine screening, and mycological research.

## MATERIALS AND METHODS

### Sample collection

Teliospore samples of smut fungi were obtained from naturally infected field-grown host plants. The fungal species studied included *Tilletia laevis*, *T. caries*, *T. controversa*, *T. indica*, *Ustilago tritici*, *U. nuda*, *U. hordei*, *Urocystis agropyri*, *Sporisorium maydis*, and *S. ehrenbergii*. All specimens were collected from symptomatic plants in various wheat and maize fields. Following collection, the samples were thoroughly air-dried and preserved in dry conditions for further processing.

### Teliospore preparation and spore wall disruption

No specific teliospore isolation procedures were applied prior to processing. Instead, the infected seeds and plant tissues were used directly. For surface sterilization, all samples were immersed in 70% ethanol for 30 seconds without removing/rupturing the husk or outer layers, followed by air drying at room temperature for approximately 15 minutes. After that, the outer layers (plant tissues/seed pericarp) were carefully removed using sterile tools and the inside spores were directly used for the next step.

For wall disruption, three approaches were tested in all four extraction methods using a sterile pestle and mortar: dry-grinding spores with carborundum at room temperature, grinding spores in liquid nitrogen without carborundum, and grinding spores with carborundum and liquid nitrogen.

For each extraction method, approximately 20 mg of ground spores were used per sample. In approaches involving carborundum, its weight was not included in this measurement.

### Murray & Thompson's DNA extraction method

Genomic DNA was extracted from teliospores using a modified cetyltrimethylammonium bromide (CTAB) method based on the protocol described by Murray and Thompson (Murray and Thompson 1980). Approximately 20 mg per sample of the ground

teliospores were transferred into sterile 2 ml microcentrifuge tubes.

Each sample was then mixed with 500 µL of pre-warmed (65 °C) CTAB extraction buffer containing 100 mM Tris-HCl (pH 8.0), 20 mM EDTA (pH 8.0), 1.4 M NaCl, and 3% (w/v) CTAB. Additionally, 4 µL of β-mercaptoethanol was added to each tube. Samples were incubated at 65 °C for 40 minutes, with brief, gentle mixing every 5 minutes to maintain suspension and ensure uniform heating.

Following lysis, 500 µL of chloroform: isoamyl alcohol (24:1) was added to each tube. Samples were vortexed vigorously and placed on a horizontal shaker for 15 minutes. Phase separation was achieved by centrifugation at 13,000 rpm for 10 minutes. Supernatant was transferred to a new tube and re-extracted with an equal volume of chloroform: isoamyl alcohol (24:1). After another 10-minute centrifugation at 13,000 rpm, supernatant was transferred to a fresh tube.

DNA was precipitated by adding an equal volume of ice-cold isopropanol, followed by incubation on ice for 15 minutes. Samples were centrifuged at 13,000 rpm for 10 minutes, and the resulting pellets were washed twice with 500 µL of 70% ethanol and centrifugation at 13000 rpm for 5 minutes. The final DNA pellets were air-dried at room temperature for 30 minutes and resuspended in 50 µL of double-distilled water (dd$H_2O$). DNA samples were stored at 4 °C overnight and then transferred to –20 °C for long-term preservation.

**Raeder & Broda's DNA extraction method**

This method was conducted using a modified Raeder & Broda protocol (Raeder and Broda 1985). Approximately 20 mg per sample of the ground teliospores were transferred into sterile 2 ml microcentrifuge tubes. To each tube, 500 µl of extraction buffer (containing 25 mM EDTA pH 8.0, 250 mM NaCl, 0.5% SDS, and 200 mM Tris-HCl pH 8.5), 350 µl of phenol (equilibrated), and 150 µl of chloroform were added. The samples were thoroughly mixed by gentle inversion for 5 minutes and then centrifuged at 13,000 rpm for 1 hour at room temperature.

The supernatant was carefully transferred to a new tube, and an equal volume of chloroform: isoamyl alcohol (24:1) was added to it. After shaking gently for 1–2 minutes, the mixture was centrifuged at 13,000 rpm for 10 minutes. The supernatant was then transferred to another clean tube, and an equal volume of ice-cold isopropanol was added to precipitate the DNA. The samples were incubated on ice for 15 minutes, followed by centrifugation at 13,000 rpm for 10 minutes.

Just like the previous method, the resulting pellets were washed twice with 70%, air-dried at room temperature for 30 minutes and resuspended in 50 µl of dd$H_2O$. DNA samples were stored at 4 °C overnight before being transferred to −20 °C for long-term preservation.

**Chelex 100 DNA extraction method**

A modified version of the Chelex 100 DNA extraction protocol was used to extract DNA from the samples (Walsh et al. 1991). Approximately 20 mg per sample of the ground teliospores were transferred into sterile 1.5 ml microcentrifuge tubes.

A volume of 200 µl of 10% Chelex 100 resin (Bio-Rad), suspended in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0), was added to each tube. The tubes were tightly sealed and incubated either in a closed-lid water bath (93–95 °C) or in a pre-warmed heat block at 96 °C for 20 minutes. To ensure homogenous temperature and suspension of the sample, the tubes were briefly and gently shaken every 7 minutes during incubation to ensure uniform heating and to prevent sedimentation of the spores. After 20 minutes, the samples were gently shaken for 10 seconds and placed on ice for 5 minutes, then centrifuged at 13,000 rpm for 10 minutes. The resulting supernatant, containing the target DNA, was carefully transferred to a new tube and stored at −20 °C for long-term preservation.

**HotSHOT DNA extraction method**

In this step, a modified HotSHOT DNA extraction protocol was used to extract DNA from the samples (Truett et al. 2000). Approximately 20 mg per sample of the ground teliospores were transferred into sterile 1.5 ml microcentrifuge tubes.

Each sample received 150 µl of alkaline lysis buffer (25 mM NaOH, 0.2 mM EDTA, pH ~12). Tubes were tightly sealed and incubated for 20 minutes in either a closed-lid water bath (93–95 °C) or a calibrated dry heat block pre-warmed to 96 °C. During incubation, the tubes were gently and briefly shaken every 7 minutes to ensure uniform heating and to prevent sedimentation of the spores. After incubation, tubes were gently shaken for 10 seconds and immediately cooled on ice for 5 minutes.

An equal volume (150 µl) of neutralizing buffer (40 mM Tris-HCl, pH 5.0) was then added to each tube and gently mixed by shaking for 10-15 seconds. Samples were centrifuged at 13,000 rpm for 10 minutes. The supernatant, containing the extracted genomic DNA, was transferred to new sterile microcentrifuge tubes and stored at −20 °C for long-term use.

**DNA quantification**

The concentration and purity of extracted DNA were measured using a Nanophotometer NP80 (Implen GmbH, Germany). For calibration, double-distilled water (dd$H_2O$) was used as the blank for DNA samples extracted with the Murray & Thompson and Raeder & Broda methods, TE buffer was used for the Chelex 100 method, and a 1:1 mixture of alkaline lysis buffer and neutralizing buffer was used for the HotSHOT method.

DNA purity was evaluated based on the absorbance ratio at 260/280 nm.

### PCR amplification

PCR amplification of fungal DNA was performed using the universal fungal primers ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′), which target the internal transcribed spacer (ITS) region of ribosomal DNA (White et al. 1990). The PCR reaction was carried out in a total volume of 10 µl per sample, containing Taq 2x master mix (Ampliqon, Denmark, 5 µl), each primer (0.5 µl), Template DNA (1 µl) and $ddH_2O$ (3 µl).

For the negative control, 1 µl $ddH_2O$ was used instead of template DNA.

PCR amplification was performed in a Bio-Rad T100™ thermal cycler using the following cycling conditions:

**Table 1.** PCR cycling conditions

| | Step | Temperature | Duration |
|---|---|---|---|
| 35x | Initial denaturation | 94 °C | 5 minutes |
| | Denaturation | 94 °C | 40 seconds |
| | Annealing | 62 °C | 50 seconds |
| | Extension | 72 °C | 90 seconds |
| | Final extension | 72 °C | 10 minutes |

### Agarose gel electrophoresis

Agarose gel electrophoresis was performed to assess the presence and approximate size of the extracted DNA and the amplified PCR products. For this purpose, 1% agarose gel was prepared using TBE buffer. Ethidium bromide (EtBr) staining was performed post-electrophoresis for visualization of DNA.

For DNA extraction quality assessment, 3 µl of each DNA sample was mixed with 2 µl loading dye before being loaded into the wells. For quality assessment of PCR products, 3 µl of each PCR product was used for loading the wells. Electrophoresis was carried out at a constant voltage of 75 V for 1 hour.

A 100 bp DNA ladder was used as the molecular weight marker. After electrophoresis, the gels were stained in EtBr solution for 15 minutes and visualized using a Vilber Lourmat TCP-20-M photo documentation system.

## RESULTS AND DISCUSSION

A comparative assessment was performed to evaluate the performance of four DNA extraction methods—Chelex 100, HotSHOT, Murray & Thompson, and Raeder & Broda—applied to ten smut fungal species belonging to the genera *Tilletia*, *Ustilago*, *Sporisorium*, and *Urocystis*. Each method was tested under three spore disruption conditions: grinding with carborundum alone, liquid nitrogen alone, and a combination of both. Nanodrop spectrophotometry and PCR electrophoresis were used to evaluate DNA yield, purity, and amplifiability. The evaluation was based on concentration, purity ratios (A260/A280 and A260/A230) and PCR amplifiability. Tables 2–5 present the Nanodrop spectrophotometry results for each method across all fungal species.

DNA yield and purity differed significantly across all these methods and there were no visible bands on gel electrophoresis of the extracted DNA, probably due to its low concentration. However, after amplification with ITS1 and ITS4 primers followed by agarose gel electrophoresis of PCR products, the presence of distinct bands of expected size (500-700 bp), confirmed the successful extraction of PCR-amplifiable DNA with all four methods.

Among these methods, the Raeder & Broda protocol provided the highest average DNA concentration (1050.55 ng/µl), followed closely by HotSHOT (951.33 ng/µl), then Murray & Thompson (918.82 ng/µl), and finally Chelex 100 (879.37 ng/µl). This trend supports the efficiency of organic solvent-based purification in the Raeder & Broda method. Notably, HotSHOT, despite being a rapid and simplified protocol, surprisingly outperformed Murray & Thompson in average yield.

**Table 2.** Nanodrop values for Murray & Thompson extraction method

| Species | Concentration (ng/µl) | A260/A280 | A260/A230 |
|---|---|---|---|
| *Tilletia laevis* | 838.45 | 1.911 | 0.889 |
| *T. caries* | 881.23 | 1.893 | 0.872 |
| *T. controversa* | 906.34 | 1.926 | 0.895 |
| *T. indica* | 912.87 | 1.943 | 0.931 |
| *Ustilago tritici* | 1007.55 | 1.926 | 0.899 |
| *U. nuda* | 930.62 | 1.911 | 0.888 |
| *U. hordei* | 940.09 | 1.905 | 0.874 |
| *Sporisorium maydis* | 859.33 | 1.934 | 0.874 |
| *S. ehrenbergii* | 1003.18 | 1.941 | 0.912 |
| *Urocystis agropyri* | 908.51 | 1.918 | 0.897 |

Purity ratios varied among methods. The Murray & Thompson and Raeder & Broda methods showed relatively balanced A260/A280 values (1.921 and 1.962, respectively), indicating minimal protein contamination. However, Chelex 100 showed a broader range, with several species exceeding 2.0

(e.g., *T. indica* = 2.604), suggesting possible RNA contamination or low protein content. HotSHOT also maintained good A260/A280 values (average: 1.869), with variability likely due to buffer residues or incomplete protein denaturation. The A260/A230 ratios were generally low across all methods (mostly 0.6–0.9), but Raeder & Broda again showed slightly higher values (average: 0.868), suggesting a more efficient removal of phenol and polysaccharides.

**Table 3.** Nanodrop values for Raeder & Broda extraction method

| Species | Concentration (ng/µl) | A260/A280 | A260/A230 |
|---|---|---|---|
| *Tilletia laevis* | 952.32 | 1.956 | 0.859 |
| *T. caries* | 986.47 | 1.938 | 0.875 |
| *T. controversa* | 1012.81 | 1.962 | 0.888 |
| *T. indica* | 1069.34 | 1.983 | 0.902 |
| *Ustilago tritici* | 1105.66 | 1.955 | 0.868 |
| *U. nuda* | 1091.14 | 1.960 | 0.851 |
| *U. hordei* | 1072.38 | 1.944 | 0.863 |
| *Sporisorium maydis* | 1018.95 | 1.981 | 0.879 |
| *S. ehrenbergii* | 1143.21 | 1.974 | 0.843 |
| *Urocystis agropyri* | 1053.26 | 1.968 | 0.847 |

**Table 4.** Nanodrop values for Chelex 100 extraction method

| Species | Concentration (ng/µl) | A260/A280 | A260/A230 |
|---|---|---|---|
| *Tilletia laevis* | 795.50 | 2.428 | 0.930 |
| *T. caries* | 691.35 | 1.961 | 0.636 |
| *T. controversa* | 834.42 | 1.968 | 0.569 |
| *T. indica* | 824.75 | 2.604 | 0.840 |
| *Ustilago tritici* | 1077.10 | 1.714 | 1.180 |
| *U. nuda* | 951.30 | 1.835 | 1.048 |
| *U. hordei* | 950.95 | 1.914 | 1.558 |
| *Sporisorium maydis* | 680.20 | 2.103 | 0.582 |
| *S. ehrenbergii* | 1204.60 | 2.157 | 0.985 |
| *Urocystis agropyri* | 783.50 | 1.889 | 0.673 |

All DNA samples from these four methods showed strong amplification of PCR products, confirmed via agarose gel electrophoresis. Figures 1–4 show the PCR amplification patterns for each method. Each gel image corresponds to one method and includes 12 wells loaded in the following order: (M) 100 bp DNA ladder, (1) *T. laevis*, (2) *T. caries*, (3) *T. controversa*, (4) *T. indica*, (5) *U. tritici*, (6) *U. nuda*, (7) *U. hordei*, (8) *S. maydis*, (9) *S. ehrenbergii*, (10) *U. agropyri* and (11) negative control using dd$H_2O$.

**Table 5.** Nanodrop values for HotSHOT extraction method

| Species | Concentration (ng/µl) | A260/A280 | A260/A230 |
|---|---|---|---|
| *Tilletia laevis* | 895.14 | 1.102 | 0.724 |
| *T. caries* | 958.23 | 1.873 | 0.704 |
| *T. controversa* | 813.81 | 2.032 | 0.627 |
| *T. indica* | 931.28 | 1.816 | 0.837 |
| *Ustilago tritici* | 782.92 | 2.301 | 0.735 |
| *U. nuda* | 1128.43 | 1.857 | 0.904 |
| *U. hordei* | 1034.83 | 1.870 | 0.691 |
| *Sporisorium maydis* | 768.69 | 1.963 | 0.813 |
| *S. ehrenbergii* | 1308.49 | 1.865 | 0.829 |
| *Urocystis agropyri* | 891.45 | 2.012 | 0.594 |

The electrophoretic profiles of PCR products were highly comparable across all methods, confirming that the extracted DNA was of sufficient quality for amplification, even in cases with low A260/A230 ratios or absence of visible DNA bands on agarose gel electrophoresis. This reinforces the critical role of spore wall disruption, particularly with carborundum-assisted grinding, which proved essential across all protocols. Grinding the spores using only liquid nitrogen proved to be insufficient in most cases.

Despite moderate A260/A230 values across all methods, the consistent PCR success implies that co-extracted inhibitors were either absent or not high enough to interfere with downstream applications under the tested conditions.

The appearance of multiple bands in PCR amplification using the universal primers ITS1/ITS4, even in single-species samples, has been previously reported. This phenomenon is mainly attributed to intragenomic heterogeneity among rDNA repeats, where non-identical ITS copies coexist within the same genome (Simon and Weiß 2008, Lindner and Banik 2011). In addition, the universal nature of ITS1/ITS4 can lead to non-specific binding to conserved but unintended regions, producing additional amplicons (White et al. 1990, Gardes and Bruns 1993). Technical factors such as high template concentration, suboptimal annealing conditions, or secondary DNA structures can further contribute to the formation of extra PCR products (Nilsson et al. 2008). Therefore, the presence

of multiple ITS bands does not necessarily indicate contamination but may reflect the inherent complexity of fungal rDNA arrays.

Ultimately, the Raeder & Broda method stands out as the most effective in terms of both yield and purity, though it requires hazardous solvents and extended processing. In contrast, HotSHOT offers a compelling trade-off, delivering high yields with minimal time and reagent usage, making it particularly suitable for rapid and routine fungal diagnostics or field-based workflows.

## Conclusion

This comparative study evaluated four DNA extraction protocols—Chelex 100, HotSHOT, Murray & Thompson and Raeder & Broda—across ten smut fungi species, including *Tilletia*, *Ustilago*, *Sporisorium*, and *Urocystis*. All four methods successfully yielded amplifiable DNA, as confirmed by ITS-PCR, despite variation in DNA yield and purity.

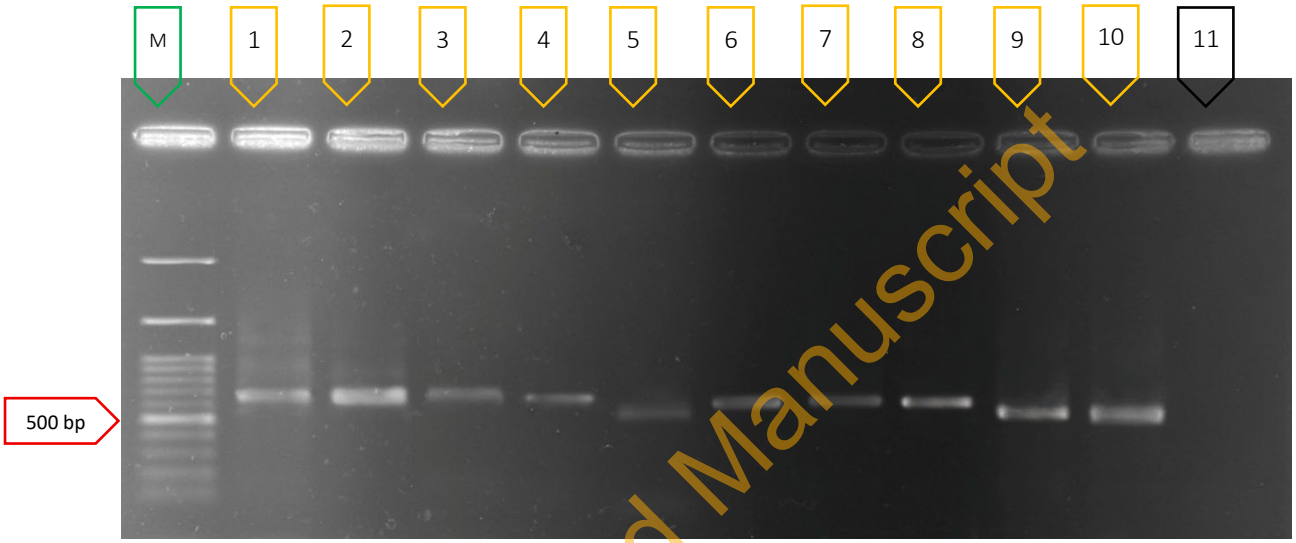


**Fig. 1.** Agarose gel electrophoresis, Murray & Thompson PCR product. (M) 100 bp DNA ladder, (1) *T. laevis*, (2) *T. caries*, (3) *T. controversa*, (4) *T. indica*, (5) *U. tritici*, (6) *U. nuda*, (7) *U. hordei*, (8) *S. maydis*, (9) *S. ehrenbergii*, (10) *U. agropyri* and (11) negative control using $ddH_2O$.

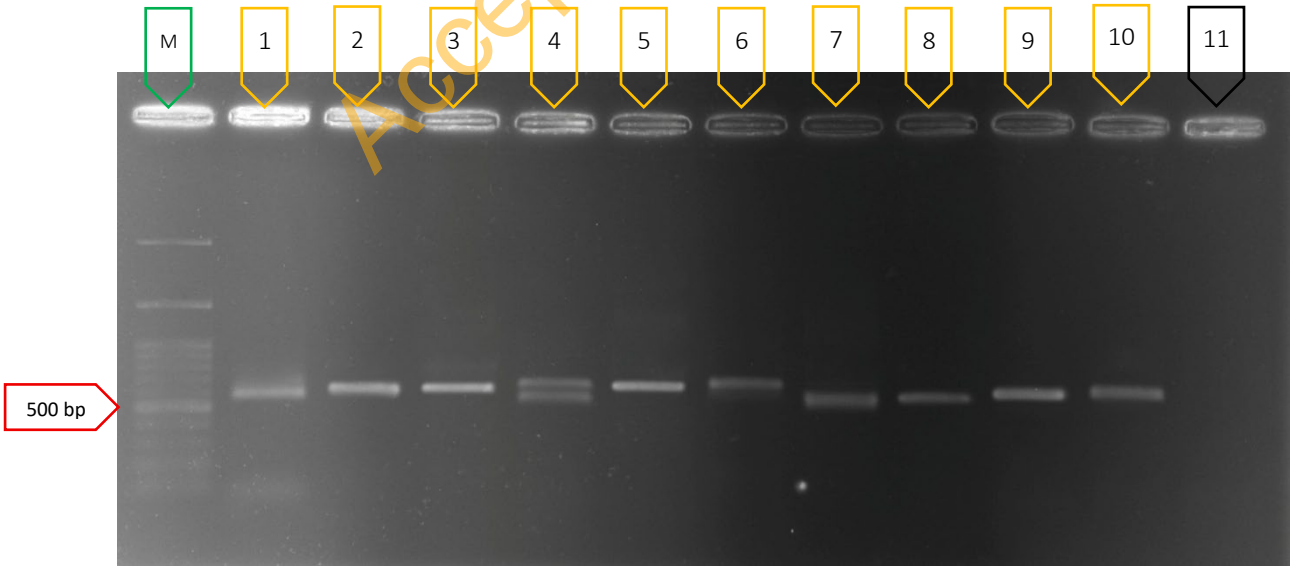


**Fig. 2.** Agarose gel electrophoresis, Raeder & Broda PCR product. (M) 100 bp DNA ladder, (1) *T. laevis*, (2) *T. caries*, (3) *T. controversa*, (4) *T. indica*, (5) *U. tritici*, (6) *U. nuda*, (7) *U. hordei*, (8) *S. maydis*, (9) *S. ehrenbergii*, (10) *U. agropyri* and (11) negative control using $ddH_2O$.

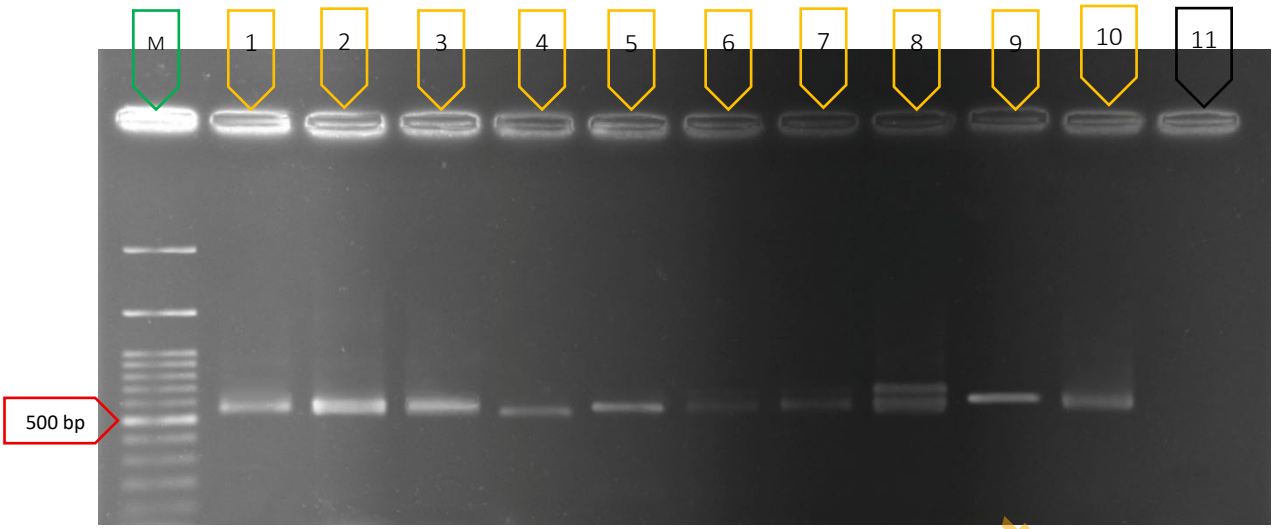


**Fig. 3.** Agarose gel electrophoresis, Chelex 100 PCR product. (M) 100 bp DNA ladder, (1) *T. laevis*, (2) *T. caries*, (3) *T. controversa*, (4) *T. indica*, (5) *U. tritici*, (6) *U. nuda*, (7) *U. hordei*, (8) *S. maydis*, (9) *S. ehrenbergii*, (10) *U. agropyri* and (11) negative control using dd$H_2O$.

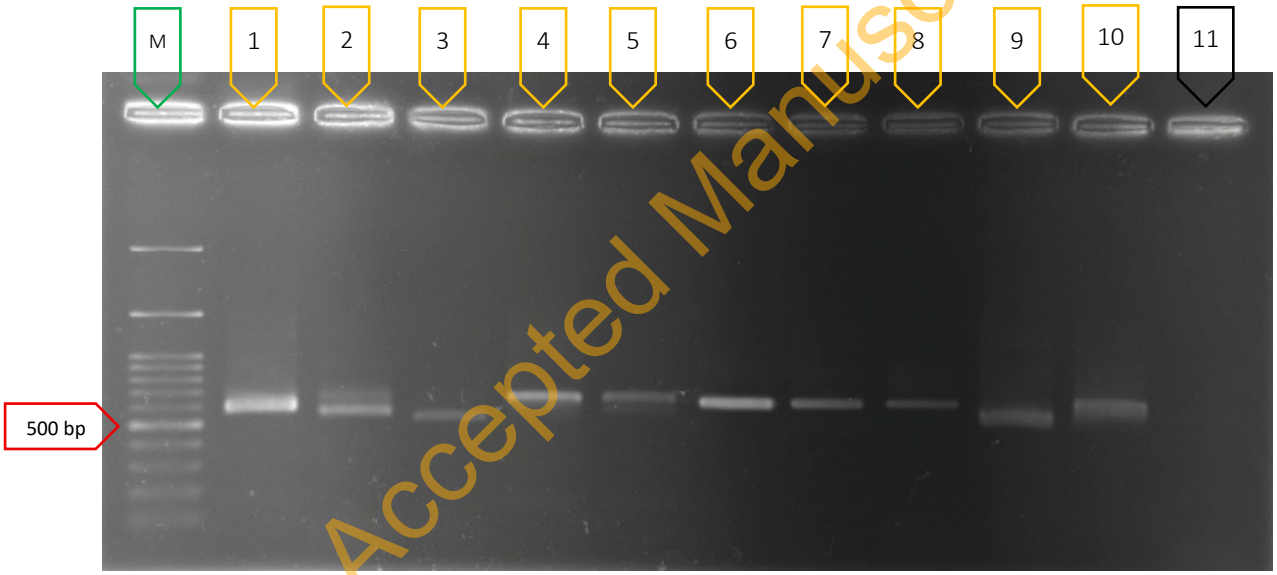




**Fig. 4.** Agarose gel electrophoresis, HotSHOT PCR product. (M) 100 bp DNA ladder, (1) *T. laevis*, (2) *T. caries*, (3) *T. controversa*, (4) *T. indica*, (5) *U. tritici*, (6) *U. nuda*, (7) *U. hordei*, (8) *S. maydis*, (9) *S. ehrenbergii*, (10) *U. agropyri* and (11) negative control using dd$H_2O$.

Among the tested methods, Raeder & Broda produced the highest average DNA concentration, likely due to strong cell lysis via SDS and efficient purification using phenol-chloroform. HotSHOT, while being a rapid and simplified protocol, also delivered a high average yield and outperformed Murray & Thompson in this respect. However, this increased yield may reflect co-purified cellular components (e.g., RNA, proteins, or degraded cell wall materials), as indicated by lower A260/A230 ratios. In contrast, Murray & Thompson-extracted DNA showed greater purity, with more stable A260/A280 and A260/A230 values, making it more suitable for downstream applications that require clean DNA, such as qPCR or sequencing. Therefore, Murray & Thompson remains the better choice when purity is prioritized over yield.

Chelex 100, although yielding the lowest DNA concentrations, remains a fast and efficient option for routine applications where rapid screening or simple PCR detection is sufficient. Its minimalistic chemistry and ease of use offer practical advantages in high-throughput workflows.

The critical role of mechanical disruption using carborundum, with or without liquid nitrogen, was evident across all protocols, especially for robust teliospores. While DNA was not visible in pre-PCR electrophoresis, all extracts produced successful and

sharp amplicons post-PCR, confirming their functional integrity.

In summary, the choice of DNA extraction method should be tailored to the specific demands of the downstream application, balancing the trade-offs between speed, yield, and purity. HotSHOT and Raeder & Broda are effective when higher DNA output is needed quickly, whereas Murray & Thompson offers superior purity, and Chelex provides simplicity with acceptable performance.

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## AUTHOR CONTRIBUTION

Bahram Sharifnabi planned, supervised and supported the research; Hossein Kargar conducted lab research and data comparison.

## DATA AVAILABILITY

The datasets used during the current study are available from the corresponding author upon request.

## DECLARATION

The authors declare no conflicts of interest.

## FUNDING

Please refer to acknowledgments.

## ETHICS APPROVAL

This article does not contain any studies with human participants or animals performed by any of the authors.

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# ارزیابی روش‌های مختلف استخراج DNA از تلیوسپور سیاهک‌ها

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## چکیده

سیاهک ها، به‌ویژه جنس های *Tilletia*، *Ustilago*، *Sporisorium* و *Urocystis* تهدیدی جدی برای غلات در جهان هستند. شناسایی مولکولی دقیق این بیمارگرها نیازمند DNA ژنومی با کیفیت بالا است. اما استخراج DNA از تلیوسپورهای آن‌ها به دلیل دیواره ضخیم و مقاوم، دشوار است. در این پژوهش چهار روش رایج استخراج DNA شامل موری و تامسون، ریدر و برودا، چلکس۱۰۰ و هات‌شات بر روی سیاهک‌های *Tilletia laevis*، *T. caries*، *T. controversa*، *T. indica*، *Ustilago tritici*، *U. hordei*، *U. nuda*، *Sporisorium maydis*، *S. ehrenbergii* و *Urocystis agropyri*، به صورت مقایسه‌ای ارزیابی شدند. تخریب مکانیکی دیواره تلیوسپورها با سه روش متفاوت انجام گرفت. کیفیت و کمیت DNA استخراج شده به کمک نانودراپ و PCR با استفاده از آغازگرهای ITS1/ITS4 بررسی شد. تمامی روش‌ها DNA قابل تکثیر با PCR تولید کردند اما بازده و خلوص در آن‌ها متفاوت بود. روش ریدر و برودا بیشترین غلظت متوسط DNA (۱۰۵۰/۵۵ ng/µl) را داشت. روش‌های هات‌شات (۹۵۱/۳۳ ng/µl)، موری و تامسون (۹۱۸/۸۲ ng/µl) و چلکس۱۰۰ (۸۷۹/۳۷ ng/µl) به ترتیب مقادیر پایین‌تری نشان دادند. روش هات‌شات بازدهی مناسبی داشت اما به دلیل خلوص کمتر احتمال وجود آلودگی داشت. در مقابل DNA حاصل از روش‌های موری و تامسون و ریدر و برودا، با توجه به نسبت های A260/A280 و A260/A230 خلوص بالاتری نشان دادند. با این وجود الکتروفورز محصولات PCR در تمامی موارد باندهای واضحی را نشان داد. این نتایج نشان می‌دهند روش موری و تامسون برای خلوص بالا، روش ریدر و برودا برای بازده بالا و روش‌های چلکس۱۰۰ و هات‌شات برای تشخیص‌های سریع مناسب هستند.

**کلمات کلیدی:** اسپور با دیواره‌ی ضخیم، چلکس، سیاهکها، هات‌شات.



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