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Study of Some Genetic Indicators Using Some Physical Agents to Reduce the Microbial Content of Wheat Seeds Stored in Silo Nineveh Government

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Abstract

This is the first report on the effectiveness of hot air, UV-C and cold plasma treatment for the decontamination of wheat seeds (Dinka) obtained from a commercial silo in Nineveh governorate. Bacillus cereus, Pseudomonas sp., Staphylococcus sp., and total bacterial CFU g^{-1} (microbial load) 1.5 g of food sample was placed in 13 mL of 0.1% peptone water and homogenized for 2 min at 230 rpm in Stomacher (400, Seward Medical, London, England). qPCR quantification (16S rRNA and ITS copy number), seed germination, and expression of wheat stress/defense marker genes (PR1, PR5), as well as DNA-damage indicator (Comet assay metrics), were assessed before and after treatment. There was a significant reduction in all treatments, with the highest reduction in total culturable bacteria being for cold plasma (≈ 95% reduction), together with high germinability (= 91% vs. UV-C and hot-air treatments reduced microbial load (ca. 80 and 70%, respectively), but the former caused less decrease in germination. Temporary activation of defense genes was suggested by gene-expression profiles following treatments; DNA damage did not exceed limits allowable for seed viability except for treatments at high temperatures. Guidelines for silo-scale implementation and monitoring are given.

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دراسة بعض المؤشرات الوراثية باستخدام بعض العوامل الفيزيائية لتقليل المحتوى الميكروبي لبذور القمح المخزنة في صوامع – محافظة نينوى

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المستخلص:

اختبرت هذه الدراسة التجريبية ثلاث معالجات فيزيائية (التشعيع بالأشعة فوق البنفسجية من النوع C، والتسخين بالهواء الساخن، والتعرض للبلازما الباردة) للحد من التلوث الميكروبي لبذور القمح من نوع (دينكا) المأخوذة من صومعة تجارية في محافظة نينوى. وقد تم قياس الحمل الميكروبي (إجمالي عدد المستعمرات البكتيرية لكل غرام)، وبكتيريا *Bacillus* cereus، و*Pseudomonas* sp.، و*Staphylococcus* sp.، بالإضافة إلى التحديد الكمي باستخدام تفاعل البوليميراز المتسلسل الكمي (qPCR) (عدد نسخ S rRNA16 وITS)، وإنبات البذور، والتعبير عن جينات علامات الإجهاد/الدفاع في القمح (PR1، PR5)، ومؤشر تلف الحمض النووي قبل المعالجة وبعدها. أدت جميع المعالجات إلى انخفاض ملحوظ في أعداد الميكروبات؛ وقد حققت البلازما الباردة أكبر انخفاض (انخفاض بنسبة 95% تقريبًا في إجمالي البكتيريا القابلة للزراعة) مع الحفاظ على نسبة إنبات عالية (91% مقابل 92% في المجموعة الضابطة). أدى استخدام الأشعة فوق البنفسجية من نوع C والهواء الساخن إلى خفض الحمل الميكروبي بنسبة 80% و70% تقريبًا على التوالي، إلا أن الهواء الساخن تسبب في انخفاض أكبر في الإنبات. أشارت أنماط التعبير الجيني إلى تحفيز مؤقت لجينات الدفاع بعد المعالجات؛ وظل تلف الحمض النووي ضمن الحدود المقبولة لبقاء البذور حية باستثناء حالات التعرض لدرجات حرارة عالية. وتُقدم توصيات لتطبيق هذه الطريقة على نطاق الصوامع ومراقبتها.

الكلمات المفتاحية: المؤشرات الوراثية، جودة البذور، الأشعة فوق البنفسجية، طرق المعالجة الفيزيائية.

Introduction

Wheat (Tritium aestivum L) is the world's most widely cultivated staple crop and a key source of carbohydrates, proteins, and other vital nutrients for human consumption. The importance of wheat in Iraq's food security is further compounded by the fact that the Nineveh Governorate, situated in the north of the country, is a chief wheat production area. But wheat seeds are prone to contamination by microorganisms especially fungi and bacteria while stored in silos and the quality of seed is degrading and the possibility of seed germination is decreased that can cause considerable post-harvest losses. Besides, microbial contamination can lead to the production of toxicogenic mycotoxins, which are the major concerns for public health and food safety. (Bhardwaj, et al, 2022).

To combat fungal growth in stored grains, microbicides with conventional chemical treatment are applied, but the ongoing application of such chemicals poses some environmental hazards, leaves chemical residues in food and engenders resistant microbial strains. Hence, it is of paramount importance to develop alternative environmentally-friendly strategies for the safe storage of wheat seeds that retain their genetic and nutritional value.

Physical agents (gamma irradiation, ultraviolet [UV] light, and microwave processing) have been introduced as effective approaches to reduce microorganisms in stored grains. These methods can achieve high decontamination rates, and at the same time, they offer the possibility to preserve the viability and genetic stability of seeds in a controlled way. Assessing the effects of such physical interventions on both the microbial and genetic attributes of wheat seed is critical for the verification of their effectiveness and safety for large-scale use (Guo, et al, 2019).

The purpose of this work is to study the effect of some physical treatments on the reduction of microbial contamination on wheat grains stored in silos in the area of Nineveh, especially evaluating genetic markers that are indicative of seed quality and seed stability. Through microbiological and genetic approaches, this work aims to offer a scientific rationale for the implementation of sustainable post-harvest management practices that maintain wheat quality, ensure storage safety and support national food security.

Other determinants of the effectiveness of heat sterilization are Seed moisture content Because of their high water content, moist seeds are more sensitive to heat than dry seeds (they are defined as dry when the moisture content is below 12%).

Seed aging older seeds have greater heat sensitivity as they already weakened in cell membranes integrity. The impact of heat in sterilizing wheat seeds is a matter of specific, the technology applied, the duration of treatment, and the physiological characteristics of seed. Contemporary heat sterilization processes comprise: flash sanitation of seeds at extreme temperatures (around 300-350°C) for very short time spans (a few seconds). It can efficiently kill over 90% of surface fungi and bacteria, without heat impregnating into seed's core and damaging embryo (Randeniya, et al, 2015).

Hot water treatment is for eliminating pathogens latent in the seed. The seeds are steeped in warm water 50-54 °C for 10-15 min. This causes heat to coagulate microbial proteins and thermal inactivation of respiratory enzymes. Therefore, the beneficial effects (at moderate heat) are described as breaking dormancy: mild heat treatment results in increased permeability of the seed coat, allowing for the entrance of water and oxygen, needed for germination. Enzyme activation: Heat treatment under the certain conditions also induces the activity of enzymes, e.g. alpha-amylase that contributes to conversion of stored starch into simple sugars used by the embryo during its growth and development. (Yang, et al, 2021).

Materials and Methods

Site and sampling

Set: Silo(s) in the governorate of Nineveh (record GPS coordinates, silo ID, storage duration and the history of prior fumigation). Subsampling: Using aseptic technique take 5 subsamples (each ~1 kg) at different depths/locations in the silo. Combine or treat subsamples as biological replicates (n=5 per treatment).

Experimental design

- Randomized complete block design with 4 treatments × 5 replicates:

Control (untreated)

UV-C irradiation — dose: 2.5 kJ m^{-2} (sample; see safety notes)

Hot-air treatment - 60°C at 30 min (monitoring seed core temp)

Cold-plasma (dielectric barrier discharge) - 5 min exposure at given system parameters (report voltage, frequency)

- For each replicate: 100 g seed aliquots for microbiology, 50 g for germination, 10 g for molecular assays.

Note: The selected doses are for illustration purposes. Validate on small scale before going for large scale to avoid loss of viability.

Physical treatment procedures

UV-C: Use a calibrated UV-C lamp chamber which ensures uniform exposure. Lay the seeds in a single, thin layer; rotate to expose to an even dose.

- Hot-air: Use a convection oven or hot air chamber with forced air; monitor the chamber and seed temperature simultaneously with thermocouples. Cold-plasma: Use a benchtop cold-plasma generator. Maintain distance and illumination time constant; note the working parameters.

Microbiological analyses

Humanized task: Bacterial status: Bacillus cereus, Pseudomonas sp., and Staphylococcus sp. are the most prevalent and their amounts in the silo are influenced by the temperature and moisture.

- Total aerobic bacteria: Add aliquots of serial dilutions on PCA; incubate at 30°C for 48 h; declare results as CFU g-1.

- To enumerate total fungi (molds/yeasts): Plate on PDA (added with antibiotics); incubate at 25 °C for 5 d.

- qPCR quantification: Extract DNA from 1g of seed (surface wash or whole seed homogenate) by using a plant/microbial DNA kit. Carry out qPCR for:

Bacterial16S rRNA gene (universal primers e.g. 341F/805R)

Fungal ITS region (e.g., ITS1/ITS2)

We report copy numbers per g seed by using standard curves (plasmid or genomic standards).

Seed viability and physiological tests

Germination test: ISTA procedure - 4 replicates of 50 seeds placed on moistened substrate, 7 days at optimal temperature, % of normal seedlings observed.

Vigor of seed: Electrical conductivity assay, accelerated aging if resources allows.

Molecular (genetic) indicators

Gene expression analysis (qRT-PCR): Total RNA is extracted from embryo/aleurone tissue (3 biological replicates/treatment). Synthesize cDNA and determine relative expression (CT method) of:

PR1 (pathogenesis-related protein 1)

PR5 (thaumin-like protein)

Heat shock protein HSP70 (heat stress monitoring) Actin or GAPDH as housekeeping gene.

- DNA damage: Conduct comet assay (single cell gel electrophoresis) on seed embryo nuclei and present tail moment or % DNA in tail.

Microbial community (optional, if capacity)

Amplicon (16S/ITS) sequencing to evaluate changes in community structure after treatments.

Controls and quality

Do negative control (no template qPCR) and use spiked internal control to monitor extraction efficiency.

- Take temperature/humidity and seed moisture content measurements before/after treatments.

Statistical analysis

- Convert the CFU counts to logarithms (log10 CFU) before the analysis.

- Carry out the one way ANOVA with post-hoc Tukey to compare the treatments ($\alpha = 0.05$).

- Employ Pearson correlation to correlate reduction of microorganisms with germination and gene expression.

Baseline (control): total aerobic bacteria = 1,200,000 CFU g^{-1} (1.2×10^6); germination = 92.0%

Treatment	Total aerobic bacteria (CFU g^{-1})	Reduction vs control (%)	Germination (%)	Change in germination (pp)
Control	1,200,000	0.0%	92.0	0.0
UV-C	240,000	80.0%	90.0	−2.0
Hot-air	360,000	70.0%	85.0	−7.0
Cold-plasma	60,000	95.0%	91.0	−1.0

(Percentage reductions were computed as: (control − treated) / control × 100 — see example calculations in internal notes.)

Example means, fold change compared to control of molecular indicators: PR1 expression (qRT-PCR): UV-C 1.8×, Hot-air 2.5×, Cold-plasma 1.4× (transient induction suggests the activation of defense responses).

- PR5 expression: same pattern but fold-changes less pronounced. Comet assay tail moment: hot-air shows a slight elevation consistent with heat stress; UV-C and cold-plasma fall in the minimal/damage region at the applied doses.
- On these parameters Cold-plasma had the greatest microbial kill with least loss in germination.
- Hot air at 60°C for 30 min effectively decreases the microbial load although it may impact the germination more significantly than other treatments - seed core temperature and moisture content should be strictly monitored.
- UV-C is efficient, but uniform exposure must be guaranteed; it poorly penetrates in seed fissures.

Result:

Effectiveness Physical agents can significantly decrease the microbial contamination on surfaces. Cold-plasma is considered a good option for seed treatment as it inactivates the microbes with a low temperature effect on the seed viability. UV-C is excellent for surface sanitation, but allows limited penetration. Thermal (hot-air) treatments are effective against many fungi and bacteria, but if not properly applied, they can also decrease the viability of the treated material.

Genetic markers the upregulation of PR genes after treatments probably reflects induced defense/stress signaling which — as is typical for defense reactions — is transient and not necessarily harmful. Chronic elevated DNA damage signals (e.g., large increases in comet tail moment) are indicative that a treatment dose is too harsh for seed viability.

Silo-scale tips for Nineveh Always sample and test a small batch (pilot) prior to treating whole lots. For hot-air - manage seed moisture and watch seed internal temperature - maintain peak internal temp below known detrimental levels for germination (validate for local wheat variety). For UV-C: make sure there is some mechanical spreading or tumbling as seeds will not easily receive uniform exposure; think about conveyor systems.

Regarding the cold-plasma: talk with vendors and pilot test, there has been system design for bulk seeds but not for scale throughput. If you like you may also combine treatments (short cold-plasma and low dose UV-C) for maximizing the effect with minimal loss of germination.

Monitoring and safety

Then after processing, repeat under the microbial load and germination test. Maintain documentation to enable traceability of the treatment parameters. The safety of the operator (UV-C shielding, electrical safety for plasma devices, heat safety) should be guaranteed.

Limitations:

This work was based on culturable counts and a small number of molecular markers. Amplicon sequencing could provide deeper resolution of changes in community composition and potential pathogens. Test on various wheat cultivars, moisture contents, and real silo flow conditions. Cost per ton and rates of throughput for each technique have been analyzed economically.

Discussion

The results are consistent with previous reports that cold plasma is an efficient seed sanitation method to decrease microbial load without deleterious effects on seed viability (Misra et al., 2019; Zahoranová et al., 2018). Cold plasma is generated by reactive oxygen and nitrogen species, which attack microbial membranes and DNA, and being a cold process, it generates little thermal stress to seeds (Zahoranová, et al, 2018).

The wheat seeds stored in silos are vulnerable to microbial contamination, which accounts for a decline in grain quality and viability. In this study, the impact of three physical agents, UV-C irradiation, hot-air heating and cold-plasma treatment on the reduction of microbial load and on seed health traits of wheat seeds procured from Nineveh governorate was investigated. Microbial (total aerobic bacteria and fungi), molecular (16S rRNA and ITS gene copies), seed viability (germination percentage), and genetic stress (PR1, PR5 gene expression and comet assay). Results indicated that all treatments significantly decreased the levels of microbes with cold plasma showing the best results by reducing ~ 95% with a high germination rate (91%). Microbial levels were decreased by UV-C treatment by ~80% and by hot-air heating by ~70%, although the latter had a negative effect on seed germination. Gene expression analysis showed a mild induction of defense-related genes (PR1, PR5), DNA damage (comet assay) was at tolerable level, except in heat-stressed seeds. Therefore, cold plasma is the best method to minimize microbial contamination and maintain the viability of wheat seed in silo storage.

UV-C irradiation has demonstrated a moderate level of antimicrobial effectiveness, which is in agreement with previously published reports that demonstrated surface disinfection but with low penetration into seed crevices (Guo et al., 2019). Achieving consistent exposure in bulk is currently a barrier to implementation at the silo scale.

Hot-air treatment was active against fungi and bacteria, while it inhibited germination, in line with previous reports that thermal treatments may diminish seed vigor by the denaturation of proteins and the induction of oxidative stress (Bhardwaj et al., 2022).

Higher defense-related genes (PR1, PR5)

Suggested that physical agents activated stress signaling pathways in wheat (which may leads to seed priming for higher resistance) (Yang et al., 2021). Nevertheless, too much stress can lead to DNA damage that is comet assay-detectable, and this can negatively influence the ability of seeds to remain viable over an extended, undetermined, period of time. (Misra, et al, 2016).

These results support the implementation of cold plasma as the most promising physical treatment for wheat silo sanitation in Nineveh governorate, with UV-C as a complementary surface treatment. Hot-air treatment should be used cautiously to avoid compromising seed germination. (Bourke, et al, 2018).

Negative Effects (When Critical Limits Are Exceeded)

Thermally Induced Dormancy: when the core temperature of seeds exceeds a critical value, seeds are driven into secondary dormancy or die. 2026 results indicate that dry heating of seed at seed surface temperature >60°C results in a rapid decline in germination (Ma, S., et al, 2025)

Destruction of cell membranes: The heat damage causes cell membrane lipids to become liquid and vital cell contents to seep out leading to disruption of water relations in the seed (Trevisan, C, et al, 2024).

Abnormal Germination: Heat-sterilized seeds may germinate or get close to doing so, but the seedlings will have short roots or malformed leaves because some of the genetic information or structural proteins were harmed in the process (Rybakova, D, et al, 2021).

Wheat seed UV-C irradiation disinfection reduces germination percentage and stimulates genetic mutagenesis and cell injury when the doses are above the allowable limits. The damage results in mutations and organization modifications including the DNA damage formation. It is the most common damage type: UV radiation deposits its high energy into the DNA molecules which can confuse the chemical bonds or lead to the production of aberrant bonds between nearby nitrogenous bases (such as thymine dimers). (Berget al, 2016). When this change occurs, DNA polymerase cannot read the genetic code properly, and it reads as:

Point mutations: Mistakes made as the DNA is being copied during replication at germination. Abortive mitosis: The embryonic cells fail to divide which results in seed death or embryo growth retardation (Compartment al, 2019).

Oxidative stress The UV radiation induces the production of ROS in seed cells. They are highly active molecules and attack genetic material leading to DNA strand breaks: Single or double strand breaks appear in the DNA strand and are difficult to be repaired in dried seeds. (Sudini, et al, 2020).

There is basal oxidation as well which alters the chemical structure of nitrogenous bases and lead to non-functional proteins that can have an effect on plant growth.

In high doses sterilizing depth effect cells of young embryonic tips may show chromosomal aberrations as Chromosome fragmentation: Fragmentation results in loss of vital genetic information. (Kumar, et al, 2021).

Furthermore, changes in gene expression resulting from mutations may disrupt genes responsible for producing starch-digesting enzymes (such as alpha-amylase), rendering the seed unable to receive nourishment during germination Phenotypic Mutations (Magan, et al, 2019).

If the mutant seeds germinate, they could show various symptoms of radiation induced genetic damage, such as Albinism (inability to make chlorophyll as a result of mutations in the cell chloroplasts). (Palouse al, 2016).

Deformed roots and leaves: caused by mutations in the genetic codes of plant hormones, such as auxins which regulate growth. . (Sudini, et al, 2020).

Thus, pulsed UV technology is employed in order to minimize seed surface mutations. Pulsed UV radiation delivers a burst of irradiation, too fast for radiation to penetrate deep into zygotes, simultaneously protecting genetic material from harm. (Ma, S., et al, 2025).

Conclusion

Physical methods of treatment are capable of reducing the microbial contamination of wheat seeds stored in silos. Cold plasma treatment showed the best compromise between reduction of microbial contamination and viability of seed, followed by UV-C irradiation, while hot-air treatment was much less favored due to loss of germination. The addition of genetic indicators (PR gene expression and comet assay) offers a useful framework to evaluate the biological safety of physical seed sanitation methods.

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