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Effects of wartime harvest delay on seed viability and grain mycobiota of maize

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The full-scale war in Ukraine has caused unprecedented disruptions to agricultural operations, leading to forced delays in maize (*Zea mays* L.) harvesting due to severe security risks, logistics constraints, and restricted field access. This study investigates the long-term impact of such delays on the physiological viability and mycobiota of maize grain during the 2025–2026 agricultural season in the north-eastern Forest-Steppe of Ukraine. Maize cobs were subjected to extreme environmental stress, remaining in the field for 148 days post-physiological maturity, experiencing prolonged exposure to harsh autumn–winter conditions, including repetitive freeze–thaw cycles and extended periods of sub-zero temperatures. Despite the retention of satisfactory grain yield (ranging from 4.74 to 5.31 t/ha) and normal physical grain characteristics, such as the 1000-grain weight (189.5–205.5 g), laboratory analysis revealed a complete loss of seed germination capacity (0%). This critical failure in viability suggests a profound disruption of embryonic tissue function and membrane integrity, likely driven by cumulative oxidative stress and dehydration caused by fluctuating environmental conditions. Furthermore, mycological analysis identified a substantial transformation of the grain's microbiological status. The fungal community was dominated by *Trichoderma viride* (50.0%), *Aspergillus flavus* (33.3%), and *Penicillium* sp. (16.7%). Statistical indices confirmed a specific, low-diversity community structure (H = 1.01; J = 0.92), which serves as an indicator of physiological weakening and degradation of the grain's protective barriers. The findings demonstrate that traditional quality metrics (typically used to assess marketable grain) are insufficient for evaluating seed suitability under conditions of prolonged field weathering. Our results suggest that such grain, although appearing physically intact, is biologically compromised and unsuitable for planting material. This study underscores the critical importance of integrating physiological germination tests and comprehensive phytosanitary screening into grain quality assessment protocols, particularly when harvesting is disrupted by large-scale conflicts or extreme climatic events. These insights are essential for ensuring future food security and agricultural resilience in regions affected by environmental and socio-economic crises.

Keywords: *Zea mays*; germination; *Trichoderma viride*; *Aspergillus flavus*; *Penicillium* sp.; low-temperature stress.

Introduction

The full-scale Russian invasion of Ukraine has become one of the most significant factors affecting agricultural production in Europe during the last decade. Military actions have disrupted agricultural activities through direct damage to farmland, destruction of infrastructure, restricted access to fields, labour shortages, fuel deficits, and logistical constraints (Datsko et al., 2025; Palchykov et al., 2026). These challenges have affected all stages of crop production, from sowing to harvesting, and have substantially altered traditional agricultural management practices (Nehrey & Trofimtseva, 2022; Hassen & Bilali, 2022). Remote sensing and satellite-based assessments indicate that large areas of agricultural land were either abandoned or remained uncultivated during the first years of the war. According to Dai et al. (2024), approximately 18 % of cropland in several eastern regions of Ukraine remained unplanted, resulting in considerable production losses of major agricultural crops. Similar findings were reported by Pechetti et al. (2023), who estimated that agricultural production in war-affected regions declined by more than 30 % compared with pre-war averages. At the national scale, war-related abandonment of agricultural land has been estimated at 2.34–2.40 million hectares, corresponding to approximately 7% of Ukraine's cropland area (Arreyndip, 2025).

Beyond reductions in cultivated area, the war has significantly affected crop productivity and harvesting operations. Similar conclusions were reached by Chen et al. (2024), who demonstrated that agricultural production losses in eastern Ukraine resulted not only from land abandonment but also from disruptions in seasonal agricultural operations. According to Kotykova et al. (2025), the combined effects of reduced cultivated area, lower crop productivity, and logistical limitations have created long-term risks for both national and global food security.

One of the most important consequences of wartime conditions has been the disruption of harvesting schedules. Several studies indicate that harvesting operations were frequently delayed because of security risks, damaged machinery, labour shortages, transportation difficulties, and restricted access to agricultural land (Lin et al., 2023; Bilali & Hassen, 2024). Similar observations were reported by Da Silva et al. (2023), whose survey of agricultural producers revealed that delayed harvesting became a common consequence of increased production costs, limited access to inputs, and security concerns associated with field operations.

As a result of these disruptions, mature maize cobs in some regions remained in the field considerably longer than recommended under conventional production systems. Prolonged exposure of grain to autumn and winter environmental conditions created a unique combination of abiotic and biotic stress factors that may influence both grain quality and its suitability for subsequent agricultural use. Among these factors, low temperatures and repeated freezing events are considered particularly important because of their potential effects on seed viability and physiological integrity.

Among the environmental factors associated with prolonged field exposure, low temperature is considered one of the most important determinants of maize seed viability. Maize is generally classified as a thermophilic crop with limited tolerance to chilling and freezing stress, particularly during critical stages of seed development and germination (Miedema, 1982). Although mature grain is typically more resistant to adverse environmental conditions than actively growing plants, prolonged exposure to sub-zero temperatures may adversely affect seed physiological status and reduce its capacity to germinate.

The negative effects of low temperatures on maize seeds are primarily associated with disturbances in cellular metabolism and membrane integrity. According to Meng et al. (2022) and Zhou et al. (2022), chilling and freezing stress induce oxidative damage, alter membrane permeability, and disrupt metabolic pathways required for normal embryo functioning. Such changes may persist even after seeds are returned to favourable environmental conditions.

The severity of freezing injury depends on several factors, among which seed moisture content plays a decisive role. Studies conducted by Neta et al. (2020) and Kostadinović et al. (2025) showed that seeds containing elevated moisture levels are considerably more susceptible to freezing damage than drier seeds. Under freezing conditions, water present within seed tissues may crystallize, causing mechanical disruption of cellular structures and irreversible damage to embryonic tissues. Therefore, seed viability and germination capacity decline substantially.

Repeated freeze–thaw cycles may further intensify these effects. During prolonged autumn–winter exposure, maize grain is frequently subjected to alternating periods of freezing and thawing, which acelerate physiological aging and increase cumulative cellular damage. Such conditions promote membrane deterioration, impair enzymatic activity, and reduce the energy reserves available for germination (Noblet et al., 2017; Meng et al., 2022). Previous studies have demonstrated that repeated temperature fluctuations can significantly reduce seed vigor even when visible grain characteristics remain unchanged.

Considerable genotypic variation in cold tolerance has been reported among maize lines and hybrids. Nevertheless, even relatively cold-tolerant genotypes exhibit reductions in germination and seedling vigor following prolonged exposure to freezing conditions (Zhao et al., 2022; Kostadinović et al., 2025). Therefore, delayed harvesting that extends grain exposure into the winter period may represent a substantial risk for maintaining seed viability, particularly when grain moisture remains elevated.

Beyond direct physiological damage, low-temperature stress may also influence the susceptibility of grain to microbial colonization. Physiologically weakened grain often becomes more vulnerable to fungal development, especially under conditions of fluctuating temperature and moisture. Consequently, prolonged autumn-winter exposure may simultaneously affect both seed viability and the composition of grain-associated fungal communities, creating additional risks for the subsequent use of grain as planting material.

The physiological weakening of grain caused by prolonged exposure to unfavourable environmental conditions may create favourable prerequisites for the development of fungal communities. During extended field weathering, maize ears are simultaneously exposed to fluctuations in temperature and moisture, which influence both grain physiology and the composition of associated microorganisms. Consequently, delayed harvesting is frequently accompanied not only by reductions in seed viability but also by changes in grain microbiological status (Krnjaja et al., 2017; Shabana et al., 2022).

Among the fungal genera most associated with maize grain are *Aspergillus*, *Penicillium*, *Fusarium*, and *Trichoderma*. Their occurrence depends on environmental conditions, grain moisture, duration of field exposure, and storage conditions (Shabana et al., 2022; Bailly et al., 2025). Surveys conducted in different agroecological regions consistently demonstrate that *Aspergillus* and *Penicillium* become dominant components of maize grain microflora during prolonged weathering and storage periods. These fungi are considered typical storage moulds capable of rapidly colonizing physiologically weakened grain and accelerating deterioration processes (Krnjaja et al., 2017).

Several studies have directly linked delayed harvesting with increased fungal contamination of maize grain. Da Costa et al. (2018) reported that postponing harvest during natural field drying significantly increased kernel rot incidence and fumonisin contamination. Similar conclusions were obtained by Katati et al. (2023), who found that prolonged field exposure promoted fungal growth, insect damage, and deterioration of grain quality. Environmental fluctuations occurring during extended exposure periods may alter fungal community structure and increase the prevalence of storage-associated fungi, thereby elevating the risk of microbial spoilage and mycotoxin accumulation.

The composition of fungal communities associated with maize grain is highly dynamic and changes in response to environmental conditions. Studies conducted by Roucou et al. (2022) and Zhang et al. (2024) demonstrated that alterations in the seed-surface microflora are accompanied by significant changes in seed vigour and physiological quality. These findings suggest that prolonged exposure of maize ears to autumn-winter conditions may result in substantial microbiological transformations even before grain enters storage facilities.

Fungal colonization is frequently accompanied by progressive deterioration of seed physiological quality. Increased abundance of storage fungi such as *Aspergillus* and *Penicillium* has been associated with reductions in germination capacity, seed vigor, and storage longevity (Wicklow et al., 1998; Zhang et al., 2024). Fungal metabolism contributes to the degradation of carbohydrates, lipids, and proteins, while the production of secondary metabolites may further compromise seed biological activity (Abbas et al., 2009). In heavily colonized grain lots, severe reductions in germination and, in some cases, complete loss of seed viability have been reported.

Although *Aspergillus* and *Penicillium* are generally regarded as indicators of grain deterioration, the presence of other fungal genera may also provide valuable information regarding the microbiological status of grain. *Trichoderma* species, for example, are widely recognized as beneficial microorganisms and biological control agents; however, they may also occur as saprophytic colonizers of grain subjected to prolonged environmental exposure (Degani et al., 2021). Their occurrence often reflects ongoing microbial succession processes within grain-associated fungal communities. Consequently, simultaneous detection of *Aspergillus*, *Penicillium*, and *Trichoderma* may indicate substantial microbiological transformation of grain resulting from prolonged exposure to environmental stressors.

Although previous studies have extensively investigated the effects of low-temperature stress on maize seed viability and germination, as well as the influence of fungal colonization on grain quality and microbiological status, information regarding the combined impact of prolonged autumn-winter field exposure under wartime harvesting delays remains extremely limited. In particular, little is known about the relationship between seed viability loss and changes in grain-associated microbiota after extended exposure of maize ears to natural winter conditions. This issue has become especially relevant in Ukraine, where military activities have frequently disrupted harvesting operations and prolonged grain exposure to environmental stressors. Therefore, evaluating both physiological and microbiological seed quality under such conditions is of considerable scientific and practical importance.

Materials and methods

The research was conducted in 2025–2026 in the north-eastern Forest-Steppe region of Ukraine. The regional climate is moderately continental, with an average annual air temperature of +7.5…+8.0 °C and an average annual precipitation of 500–600 mm. The soil cover consists mainly of grey forest loamy soils and podzolized chernozems, which are characterized by a sufficient level of natural fertility and are suitable for grain maize cultivation.

The object of the study was maize (*Zea mays* L.) grain of the DKS 3402 hybrid, grown in commercial fields following sunflower. The crop was sown between May 1 and May 9, 2025, in accordance with the farm's standard cultivation technology. During sowing, complex mineral fertilizer (nitroammophoska, $N_{16}P_{16}K_{16}$) was applied at a rate of 100 kg/ha of physical mass. The total area of the fields used for the research was 257 hectares.

The study was designed with a three-fold replication for each of the four commercial maize fields (№ II, III, IV, and VI) resulting in a total of 12 experimental units, which were treated as independent production replicates. All fields were characterized by identical hybrid composition and similar technological cultivation conditions.

The research was conducted in compliance with relevant institutional, national, and international guidelines for agricultural research. The experimental procedures, including field sampling and laboratory analysis, were carried out according to standard ethical practices for plant science research. The study adheres to the principles of transparency and reproducibility, as outlined in the Research and Reporting Guidelines provided by the National Library of Medicine. No sensitive biological or human subjects were involved, and no specific ethical approval from an Institutional Review Board was required for this type of plant-based experimental study.

Due to restrictions related to the martial law in Ukraine and logistical difficulties, harvesting was not performed within the optimal agrotechnical timeframe. As a result, maize cobs remained on the plants after reaching physiological maturity throughout the autumn-winter period and were subjected to prolonged exposure to natural environmental factors. During this period, the grain was exposed to low temperatures, repeated freeze–thaw cycles, precipitation, and fluctuations in air humidity. According to meteorological data, the minimum air temperature during the time the ears remained in the field reached –22 °C, while the total number of days with temperatures below 0 °C was approximately 69.

The research was designed as a field observation of the effects of natural autumn-winter conditions on maize grain quality following a forced harvest delay. The primary objective was to assess changes in seed sowing qualities and the characteristics of fungal microbiota development after prolonged field exposure of the ears.

During harvesting, crop productivity indicators were determined, including grain yield, grain moisture, and 1000-grain weight. The yield was adjusted to a standard moisture content of 14%. Grain moisture was determined using generally accepted industrial quality control methods.

Prior to setting up the experiment, laboratory equipment was sterilized and the working environment was prepared. Work surfaces and instruments were treated with 96% ethyl alcohol (Bio-Tech, Kyiv, Ukraine). The laboratory room was pre-irradiated with a germicidal lamp (Model SP-T5-UV, 8W, China) for 60 minutes. Petri dishes and metal instruments were sterilized in a drying oven (Sterilizer GPO-50, UOSLAB, Ukraine) at 130 ± 2 °C for 60 minutes.

To assess sowing qualities, average grain samples were formed separately from each production field. Laboratory germination was assessed by placing 100 seeds from each replicate (divided into five Petri dishes with 20 seeds each) onto sterile filter paper moistened with distilled water. The dishes were incubated in a climate chamber at 25 ± 2 °C. Seed germination was recorded after 7 days, with a seed considered germinated upon the emergence of both the primary root and coleoptile.

Fungi were identified based on a complex of macro- and micromorphological characteristics, as well as by taking into account modern approaches to studying maize seed mycobiota (Shabana et al., 2022). Seeds were plated on potato-glucose agar (LLC "Chemlaborreaktiv", Brovary, Ukraine), after which the Petri dishes were incubated at 25 ± 2 °C for 7 days.

After the completion of incubation, the number of colonies was counted, the frequency of occurrence of individual fungal taxa was determined, and their identification was carried out based on a combination of macro- and micromorphological features. To clarify the species identity, temporary slides were prepared, and the structural features of the mycelium, conidiophores, and sporulation were examined using light microscopy (Levenhuk 870T, USA).

To evaluate the structure of the grain's fungal mycobiota, species diversity and community evenness indices were additionally calculated (Pielou et al, 1966). Species diversity was assessed using the Shannon diversity index, which was determined by the following formula:

$$H = -\Sigma(p_i \times \ln p_i),$$

where H is the Shannon index; p_i is the proportion of colonies of the i-th taxon in the total number of isolates; ln is the natural logarithm.

To evaluate the uniformity of taxon distribution, Pielou's evenness index was used:

$$J = H / \ln(S),$$

where J is Pielou's evenness index; H is the Shannon index; S is the number of detected taxa.

Statistical processing of the research results was performed using variational statistics and analysis of variance methods with the Statistica 10.0 software package (StatSoft Inc., Tulsa, USA). For quantitative indicators, mean values and their standard errors were determined. The significance of differences between means was assessed using Tukey's HSD test at a significance level of P < 0.05.

Results

Despite the prolonged field exposure of maize cobs after reaching physiological maturity, the plants produced grain yields consistent with regional cultivation conditions. Analysis of variance indicated statistically significant differences among the studied fields for all productivity and physical grain characteristics (P < 0.001, Table 1).

Grain yield across the studied commercial fields ranged from 4.74 to 5.31 t/ha. The highest yield was recorded in field I (5.31 ± 0.03 t/ha), which significantly outperformed all other experimental units. Fields II and IV exhibited the lowest productivity, with values of 4.80 ± 0.03 and 4.74 ± 0.05 t/ha, respectively; these values did not differ significantly from each other according to Tukey's HSD test.

Grain moisture at the time of harvesting ranged from 21.70% to 23.20%. The lowest moisture content was observed in field I (21.70 ± 0.17%), while the highest was recorded in field IV (23.20 ± 0.26%). Despite the prolonged field exposure of the ears, the grain maintained high moisture content, necessitating post-harvest drying before storage.

The 1000-grain weight varied significantly among fields, ranging from 189.57 to 205.50 g. The highest 1000-grain weight was recorded in field IV (205.50 ± 0.66 g), while the lowest was observed in field I (189.57 ± 0.47 g). Statistical analysis confirmed that the differences in grain mass were significant across the experimental sites.

Table 1
Productivity indicators and physical characteristics of maize grain after prolonged field exposure (x ± SD, n = 3)

Field	Yield, t/ha (at 14% moisture)	Grain moisture at harvest time, %	1000-grain weight, g
I	5.31 ± 0.03^{a}*	21.70 ± 0.17^{a}	189.57 ± 0.47^{c}
II	4.80 ± 0.03^{c}	21.90 ± 0.20^{a}	192.80 ± 0.46^{b}
III	5.20 ± 0.03^{b}	22.20 ± 0.10^{a}	191.70 ± 0.36^{b}
IV	4.74 ± 0.05^{c}	23.20 ± 0.26^{b}	205.50 ± 0.66^{a}

Note: values followed by different letters within the same column are significantly different at P < 0.05 according to Tukey's HSD test.

Laboratory germination tests revealed a complete loss of seed viability across all studied samples (Table 2). Following a 7-day incubation period on a nutrient medium, no seed germination, primary root development, or coleoptile formation was recorded for any of the tested samples (n = 400 total seeds).

Table 2
Laboratory germination of maize grain after prolonged field exposure during the autumn-winter period (n = 4)

Field	Number of seeds tested, pcs.	Number of germinated seeds, pcs.	Laboratory germination, %
I	100	0	0
II	100	0	0
III	100	0	0
IV	100	0	0

The meteorological conditions during the period from November 2025 to March 2026 (148 days) were characterized by persistent exposure to sub-zero temperatures (Table 3). Out of the total duration, 69 days (46.6%) were recorded with air temperatures below 0 °C. The absolute minimum temperature during the observation period was −22 °C, recorded in February 2026. Furthermore, the analysis of the daily temperature regime identified 12–15 freeze–thaw cycles, defined by transitions of the daily mean temperature across the 0 °C threshold.

To evaluate the phytosanitary status of the seeds, mycological analysis was performed using the potato-glucose agar (PGA) incubation method. In all grain samples, incubation resulted in the intensive growth of microscopic fungal colonies, concurrent with the complete absence of seed germination (Fig. 1).

As a result of the mycological analysis, three dominant isolated fungal taxa were identified: *Trichoderma viride* Pers., 1794, *Aspergillus flavus* Link, 1809, and *Penicillium* sp. The largest share in the mycobiota structure was accounted for by *Trichoderma viride* – 50.0% of the total number of detected colonies (Table 4). The share of *Aspergillus flavus* was 33.3%, while representatives of *Penicillium* sp. were detected in 16.7% of cases. The distribution of 12 isolated colonies among the identified taxa was analyzed using the Chi-square goodness-of-fit test. The observed frequencies did not differ significantly from the theoretical uniform distribution ($\chi^2 = 2.00$, df = 2, P = 0.368). Ecological indices indicated a Shannon diversity index (H) of 1.01 and a Pielou evenness index (J) of 0.92, suggesting a relatively balanced, though small, mycobiota complex.

The Shannon diversity index (H = 1.01) and Pielou's evenness index (J = 0.92) characterize the fungal community as having low taxonomic diversity yet a relatively uniform distribution of the identified taxa. These indices reflect the formation of a specialized fungal community developed during prolonged autumn–winter field exposure. While the grain maintained its morphological integrity, the complete loss of viability and significant fungal colonization indicate a substantial degradation of its physiological and sanitary status.

Table 3
Temperature conditions during the period the maize ears remained in the field after reaching physiological maturity (November 2025 – March 2026)

Month	Average temperature, °C	Minimum temperature, °C	Number of days with t < 0 °C
November 2025	+8.0	+2	0
December 2025	+1.5	−7	9
January 2026	−5.0	−14	29
February 2026	−4.2	−22	23
March 2026	+4.5	−3	8
Mean	+0.9	−22	69
Duration of ears in the field, days	148		
Total number of days with t < 0 °C	69		
Share of frosty days, %	46.6		
Absolute minimum temperature, °C	−22		
Estimated number of freeze–thaw cycles	12–15*		

Note: determined by the number of transitions of the average daily temperature through 0 °C.

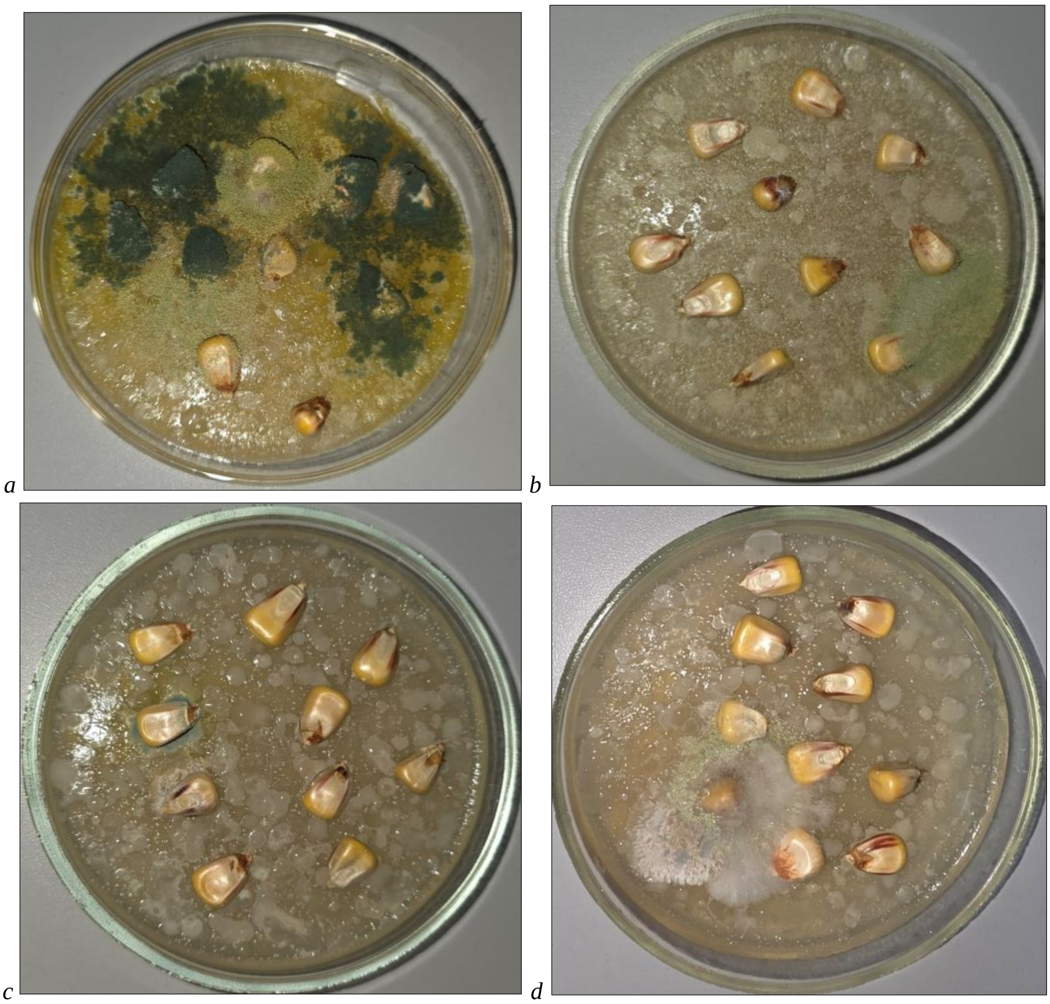

Fig. 1. Absence of maize grain germination and fungal colonization after 7 days of incubation on potato-glucose agar: *a*–*d* – grain samples from fields I, II, III, and IV, respectively

Table 4
Species composition of the fungal mycobiota of maize grain after prolonged field exposure of the cobs (n = 12)

Fungal species	Number of colonies	Share, %
Trichoderma viride	6	50.0
Aspergillus flavus	4	33.3
Penicillium sp.	2	16.7

Note: total number of detected taxa = 3; total number of representative isolates = 12; Shannon diversity index (H) = 1.01; Pielou's evenness index (J) = 0.92.

The mycobiota was dominated by *Trichoderma viride*, which exhibited rapid growth, dense mycelium, and intense dark green sporulation (Fig. 2). Microscopic analysis confirmed characteristic branched conidiophores, numerous unicellular conidia, and septate mycelium. The prevalence of *T. viride* suggests high competitive ability and rapid colonization of the weakened embryonic tissues of the maize caryopsis.

The morphological features of the dominant taxon, *T. viride*, are shown in Figure 2. The colonies were characterized by intensive growth, a dense green sporulating coating, and a well-developed septate mycelium. Microscopic analysis revealed the species-specific branched conidiophores, numerous unicellular conidia, and well-developed septate mycelium. Structures similar to chlamydospores, which often form in *Trichoderma* cultures during prolonged cultivation, were also observed. The high frequency of *T. viride* detection indicates its ability to actively colonize plant substrates after prolonged exposure to high humidity and fluctuating temperatures. Although members of the genus *Trichoderma* are typically viewed as antagonists of many phytopathogenic fungi, their mass development in this case serves as an indicator of profound changes in the microbiological status of the grain after wintering in the field.

Representatives of *Aspergillus flavus* formed characteristic yellowish-green colonies with abundant sporulation (Fig. 3). Under the microscope, typical spherical vesicles covered with phialides and chains of conidia were observed. The presence of this species warrants special attention due to its potential ability to produce aflatoxins and colonize grain under high humidity conditions.

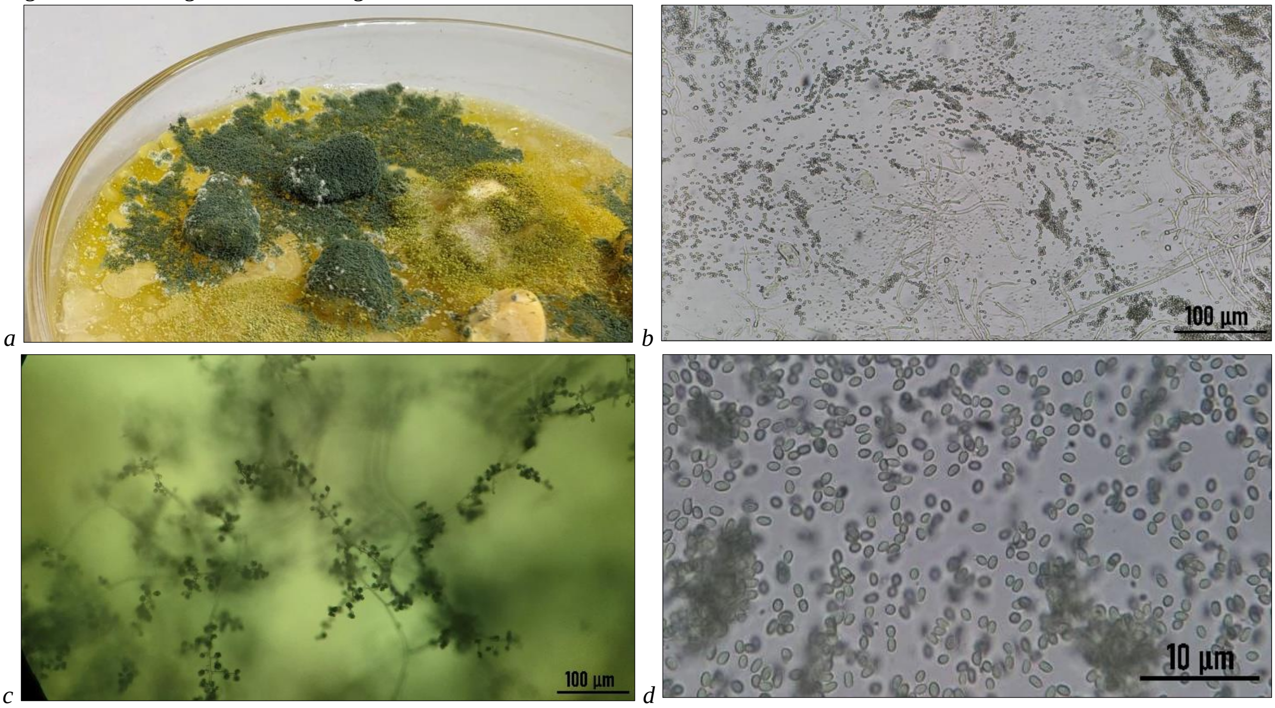

Fig. 2. Morphological features of *Trichoderma viride* isolated from maize grain after prolonged field exposure of the ears: *a* – *Trichoderma viride* colony on potato-glucose agar; *b* – branched conidiophores; *c* – conidia and phialides; *d* – septate mycelium and chlamydospores

Aspergillus flavus was the second most prevalent representative of the mycobiota, accounting for 33.3% of the total number of identified isolates. The colonies were characterized by a yellowish-green colour and abundant sporulation (Fig. 3). During microscopic analysis, spherical vesicles typical of the species, covered with phialides, and numerous chains of conidia forming characteristic capitate sporulation structures were observed.

Representatives of *Penicillium* sp. accounted for 16.7% of the total number of colonies. The colonies had an olive-green colour and a velvety surface. Microscopically, characteristic penicillate sporulation with branched conidiophores and numerous chains of small unicellular conidia was detected (Fig. 3).

In conclusion, *Trichoderma viride*, *Aspergillus flavus*, and *Penicillium* sp. dominated the composition of the grain's fungal mycobiota. The identification of these fungi confirms the active colonization of the grain during its prolonged stay in field conditions. At the same time, the complete absence of seed germination in all studied samples indicates a loss of sowing qualities regardless of the external state of the kernels.

Discussion

The results obtained in this study indicate that prolonged field exposure of maize ears during the autumn–winter period did not prevent the formation of grain yield and acceptable physical grain characteristics. Grain yield ranged from 4.74 to 5.31 t/ha, while the weight of 1000 kernels varied between 189.5 and 205.5 g, suggesting that kernel filling and grain development were largely completed before the onset of winter conditions. However, despite the preservation of these productivity indicators, the harvested grain exhibited a complete loss of germination capacity, indicating severe deterioration of seed viability.

The study was conducted under actual wartime agricultural conditions, where harvesting delays resulted from operational constraints rather than experimental design. Consequently, the obtained results reflect real production scenarios encountered by farmers in conflict-affected regions and provide practical evidence of the biological consequences of prolonged autumn–winter field exposure of maize ears.

The complete absence of seedling emergence observed in the present study indicates that prolonged autumn–winter field exposure caused severe deterioration of seed viability despite the preservation of acceptable grain appearance and physical characteristics. Previous studies have demonstrated that freezing stress disrupts membrane integrity, induces oxidative damage, alters cellular metabolism, and impairs physiological processes essential for embryo functioning and germination (Ikhajiagbe et al., 2020; Boughazi et al., 2024). Prolonged exposure to sub-zero temperatures may result in irreversible injuries to embryonic tissues, leading to a substantial decline in germination potential even when external grain morphology remains largely unaffected (Sun et al., 2020; Hu et al., 2025).

The deterioration observed in the present investigation was likely intensified by repeated freeze–thaw cycles that occurred during the 148-day period of field exposure. Such temperature fluctuations are known to promote cumulative cellular damage through membrane destabilization, dehydration stress, and disruption of intracellular structures, particularly under conditions of fluctuating grain moisture content (Moradtalab et al., 2018; Waqas et al., 2021). Embryonic tissues are considered among the most vulnerable components of maize grain under low-temperature stress, and previous histological and physiological studies have shown that freezing injury may impair meristematic activity, enzymatic functioning, and energy metabolism required for successful germination (He et al., 2019; Božić et al., 2021). Therefore, the complete loss of germination recorded in this study is most likely the result of extensive freeze-induced damage accumulated during prolonged winter field exposure.

In addition to physiological deterioration, fungal colonization may have contributed to the observed loss of seed viability. The detection of *Aspergillus*, *Penicillium*, and *Trichoderma* indicates substantial microbiological transformation of grain during field exposure. Previous studies have reported that *Aspergillus* and *Penicillium* frequently colonize physiologically weakened grain and accelerate deterioration processes through enzymatic degradation of storage compounds and production of secondary metabolites (Aminu & Keta, 2021; Goko et al., 2021). Similar relationships between fungal colonization and reduced seed vigour have been documented in maize grain subjected to prolonged environmental exposure (Popoola & Okungbowa, 2021).

The simultaneous occurrence of freezing injury and fungal colonization suggests that seed deterioration resulted from the combined action of abiotic and biotic stress factors rather than from a single cause. Grain exposed to prolonged winter conditions likely experienced progressive physiological weakening, creating favourable conditions for fungal establishment and further deterioration. This interpretation agrees with observations reported by Musa et al. (2026), who highlighted the importance of interactions between environmental stress and grain-associated microbial communities in determining seed quality outcomes.

Overall, the present study demonstrated a pronounced discrepancy between the physical condition of maize grain and its physiological viability. Despite maintaining acceptable productivity indicators and marketable appearance, all examined grain samples completely lost their germination capacity after prolonged autumn–winter field exposure. Under conditions associated with wartime harvesting delays, visual assessment and conventional grain quality parameters may therefore substantially overestimate the suitability of grain for subsequent use as planting material. These findings highlight the importance of integrating physiological and microbiological analyses into seed quality assessment when harvest operations are disrupted by extreme environmental or socio-economic conditions.

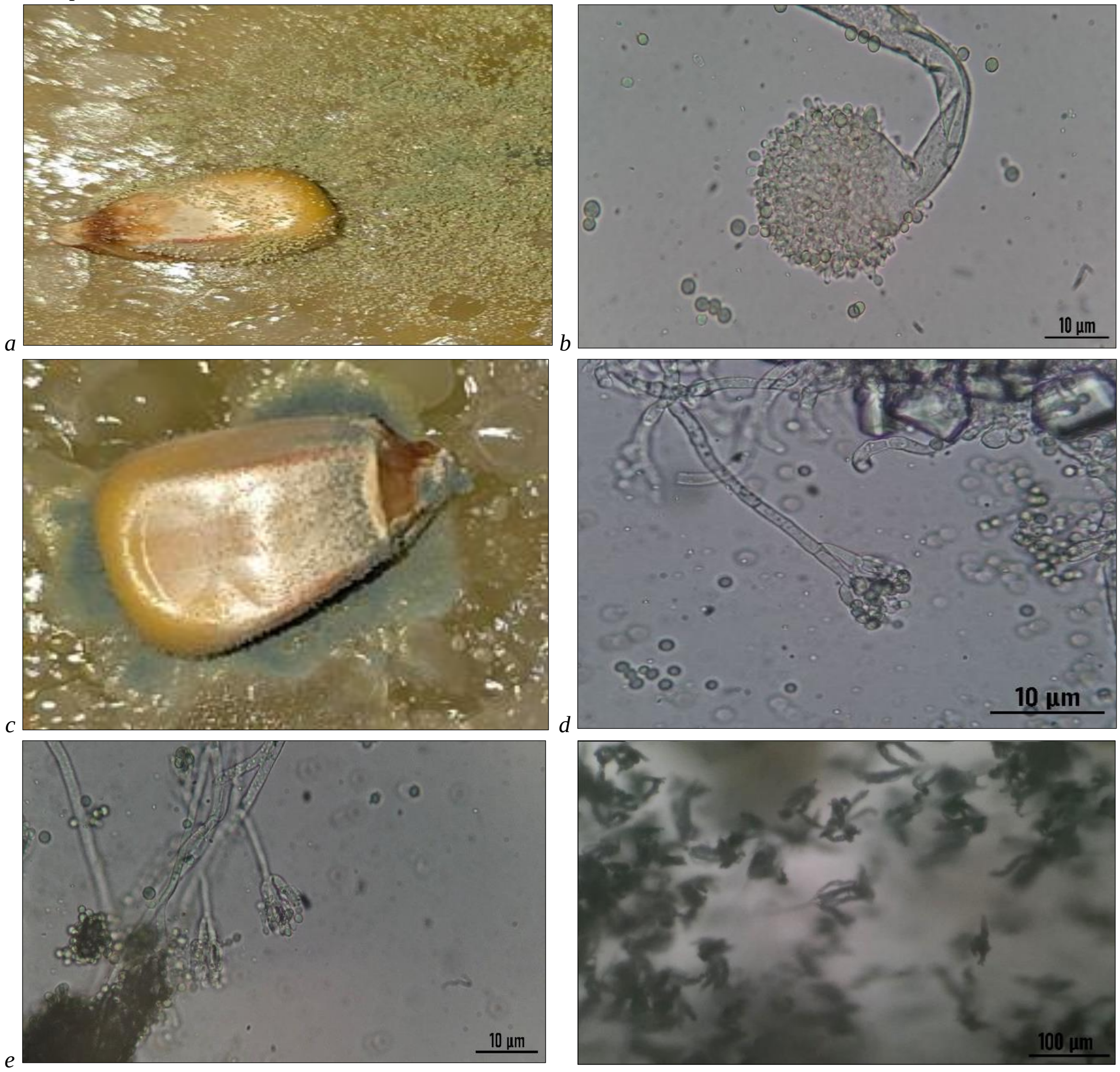

Fig. 3. Morphological features of *Aspergillus flavus* and *Penicillium* sp. isolated from maize grain after prolonged field exposure of the ears: *a* – *Aspergillus flavus* colony on potato-glucose agar; *b* – *Aspergillus flavus* conidiophore and vesicle; *c* – *Penicillium* sp. colony; *d* – *Penicillium* sp. penicillate sporulation; *e* – *Penicillium* sp. conidial chains

Conclusion

The forced delay in maize harvesting under martial law conditions resulted in cobs remaining in the field for 148 days post-physiological maturity. During this interval, the grain was subjected to extreme environmental stress, including temperatures as low as –22 °C and 12–15 freeze–thaw cycles.

Despite this prolonged exposure, the grain maintained acceptable physical and productivity metrics, with yields ranging from 4.74 to 5.31 t/ha and a 1000-grain weight of 189.5–205.5 g. However, these traditional quality indicators masked a critical failure in physiological integrity: laboratory germination was 0% across all samples. This complete loss of viability was further compounded by a shift in the mycobiota, dominated by *Trichoderma viride* (50.0%), *Aspergillus flavus* (33.3%), and *Penicillium* sp. (16.7%), characterized by low taxonomic diversity (H = 1.01) but a uniform distribution of these dominant taxa (J = 0.92).

These findings demonstrate that under conditions of prolonged autumn-winter field exposure, external grain appearance and traditional productivity metrics are insufficient criteria for assessing seed suitability. We conclude that laboratory germination and mycological screening are essential to prevent the use of compromised grain as planting material. Future research should prioritize the quantification of mycotoxins in such grain lots to evaluate their safety for feed and industrial processing, further expanding the understanding of how biotic and abiotic stressors intersect in conflict-impacted agricultural zones.

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