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INFLUENCE OF AGROTECHNICAL AND VARIETAL FACTORS ON BIODIVERSITY OF FUNGI COLONIZING AMARANTH SEEDS

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ABSTRACT

Amaranth seeds are characterized by a very high nutritional value, which depends on various environmental factors and variety. For this plant, it is crucial to maintain the appropriate post-harvest quality of the seeds, particularly in terms of fungal infections. The aim of this study was to evaluate the impact of increasing NPK doses, row spacing, and variety on the biodiversity of fungi colonizing amaranth seeds. For this purpose, a 3-year field experiment (2016–2018) was carried out involving the cultivation of two varieties – 'Rawa' and 'Aztek' – under the soil and climatic conditions of southeastern Poland. The experiment demonstrated that most of the analyzed research factors (NPK fertilization, row spacing, and variety) had a significant impact on the health status of amaranth seeds. Increasing NPK fertilization led to higher seed colonization by *Alternaria alternata*. Row spacing affected both the total number of fungal colonies and the population of *Aspergillus brasiliensis* on the seeds. In this regard, amaranth cultivation was more favorable with wide row spacing than narrow row spacing. The variety significantly influenced seed germination after harvest, and the total number of fungal colonies on the seeds, particularly of *Aspergillus brasiliensis* and *Alternaria alternata*. The variety 'Rawa' was characterized by lower seed germination capacity and was more frequently populated by pathogenic fungi, which negatively affected seed quality compared to the variety 'Aztek'. Considering the production of seeds free of post-harvest biological contamination in southeastern Poland, it is advisable to cultivate the variety 'Aztek' in a wide row spacing system.

Keywords: amaranth, seed health, fungal biodiversity, *Aspergillus brasiliensis*, *Alternaria alternata*

INTRODUCTION

Amaranth is classified as a pseudocereal. Its seeds are highly nutritious and can serve as a substitute for native cereal crops [Skwaryło-Bednarz et al. 2020]. Under the soil and climatic conditions of Poland it shows high pest resistance. Maintaining high post-harvest seed health poses the greatest challenge for amaranth growers. This requires the rapid drying of harvested seeds to a moisture content of 11–13% to prevent the development of fungi, particularly of the genera *Aspergillus*, *Alternaria*, *Penicillium*, and *Fusarium*, which could contaminate the seed yield with mycotoxins hazardous to animal and human health [Krasowska 2022]. Currently, since there are no registered chemical agents for the protection of amaranth, only non-chemical solutions are utilized for this purpose. Amaranth is characterized by fairly high resistance to fungi inhabiting agricultural ecosystems. Fungi of the genus *Alternaria*, transmitted through seeds, cause leaf spot diseases [Blodgett and Swart 2002, Pusz 2009b, Pusz 2009c]. *Aspergillus brasiliensis* (previously known as *A. niger*) and *Aspergillus flavus*, both widely distributed in nature, are also frequently found on amaranth seeds. They often

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develop as hyphae on the seeds, which subsequently form spores, enabling their rapid spread [Oyeyiola 2002, Pusz 2009a]. Fungi of the genus *Aspergillus* also secrete numerous mycotoxins, particularly the poisonous and carcinogenic aflatoxins (AFB1, AFB2, AFG1, AFG2), ochratoxin A, and over 50 other secondary metabolites. These can affect consumer health and cause infection symptoms in plants [Klich 2007]. Another fungus, *Alternaria alternata*, produces many secondary metabolites, among which alternariol (AOH), methyl ether of alternariol (AME), altenuene (ALT), alternariol toxins (ATX), and tenuazonic acid are among the most well-studied [Noelting et al. 2016, Escrivá et al. 2017]. Fungi from the genus *Fusarium* are highly dangerous to amaranth crops. Particularly harmful to various amaranth species are *Fusarium oxysporum, F. avenaceum, F. equiseti, F. solani* and *F. sambucinum* [Blodgett et al. 2004, Pusz and Pląskowska 2012]. All *Fusarium* species produce toxic mycotoxins, including deoxynivalenol (DON), nivalenol (NIV), moniliformin (MON), zearalenone (ZEA), trichothecenes, and fumonisin B1 (FB1). The biosynthesis mechanisms of these mycotoxins are influenced by various environmental conditions such as pH, temperature, humidity, and nitrogen availability [Bakker et al. 2018, Oufensou et al. 2021]. Many of these mycotoxins, such as fumonisins and trichothecenes, are thermally stable and cannot be deactivated by cooking [Perincherry et al. 2019]. Contamination with mycotoxins of fungi from the genus *Penicillium* is also found on many stored crops. *Penicillium expansum* produces ochratoxin A and patulin on stored fruits, both of which are harmful to consumers [Cabañes et al. 2010, Oteiza et al. 2017].

To date, few field studies have been carried out worldwide or in Poland on the impact of fertilization with varying doses of macronutrients on pseudocereal crops, which are generally considered to respond well to NPK fertilization. The objective of these studies is to determine the most appropriate fertilizer doses to obtain optimal post-harvest amaranth seed health in the context of potential mycotoxin contamination.

The selection of the research problem enabled the precise formulation of the study's objective, i.e., to evaluate the impact of varied NPK fertilization, row spacing, and variety on the post-harvest health status of amaranth seed yield.

The null hypothesis (H0) posited that NPK fertilization and row spacing would not affect the post-harvest health of amaranth seeds of the varieties 'Rawa' and 'Aztek' cultivated under the edaphoclimatic conditions of southeastern Poland.

The alternative hypothesis (H1) was to prove that increasing doses of NPK fertilization, and row spacing would affect the post-harvest health of amaranth seeds of the varieties 'Rawa' and 'Aztek' grown under soil and climate conditions of southeastern Poland.

MATERIALS AND METHODS

Field experiment description

The field experiment was conducted between 2016 and 2018 on an individual farm located in Bodaczów (50°71'N, 23°04'E) near Zamość, in southeastern Poland. The experiment was set up using a split-plot method in a randomized block design with three replications. The plot size was 10 m² (2 m × 5 m). The experiment was established on brown soil derived from loess. Each year, before setting up the experiment, soil samples were collected for chemical analysis to determine the content of phosphorus (P), potassium (K), magnesium (Mg), organic matter and soil pH. The mineral content of the soil, i.e., P, K and Mg, was high, while the pH was neutral (pH in l mol KCl dm^{-3} – 6.8 (2015), 6.9 (2016), and 6.8 (2017)).

The forecrops for amaranth were spring barley in 2016, spring wheat with white mustard as an intercrop, and winter wheat in 2018. The experiment included the following variables: NPK fertilization (n = 4), row spacing (n = 2), varieties (n = 2), and replicates (n = 3); total n = 48:

I. Variations of NPK fertilization (kg ha^{-1}):
   1. Control – 0.0 N, 0.0 P_2O_5, 0.0 K_2O – NPK0,
   2. 80.0 N, 50.0 P_2O_5, 50.0 K_2O (80 N, 22 P, 41.5 K = 143.5 NPK) – NPK1,
   3. 110.0 N, 70.0 P_2O_5, 70.0 K_2O (110 N, 30.8 P, 58.1 K = 198.9 NPK) – NPK2,
   4. 140.0 N, 90.0 P_2O_5, 90.0 K_2O (140 N, 39.6 P, 74.7 K = 254.3 NPK) – NPK3.

II. Row spacing:
   1. 30 cm,
   2. 55 cm.

III. Two amaranth varieties:
   1. 'Rawa',
   2. 'Aztek'.

All tillage and cultivation practices were carried out in accordance with generally accepted principles

of proper agronomy. Seeds of two amaranth varieties – 'Rawa' and 'Aztek' with full utility value, were sown at a rate of 2.0 kg ha^{-1} using a plot seeder (Tool Carrier 2700) in row spacings of 30 cm for narrow row sowing and 55 cm for wide row sowing. Sowing was carried out in the third decade of May (2016 and 2017) or in the first decade of June (2018). In each year of the field experiment, certified seed material was provided free of charge by company "Szarłat" M. and W. Lenkiewicz sp.j.

Every year, the experimental field underwent two rounds of manual and mechanical maintenance to remove excessive weeds from the plantation. The experiment did not require annual protection against agrophages, i.e., pathogens and pests. This absence posed challenges due to the limited availability of plant protection products specifically designed for amaranth.Seeds were harvested in the third decade of October (2016) and the first decade of November (2017, 2018). After harvesting, the seeds had a high moisture content, which required rapid drying to maintain their quality and health parameters. In 2016, the seed moisture content directly after harvesting and threshing was 28.9%, 21.3% in 2017, and 24.4% in 2018. The seeds were dried each year to a moisture content of approximately 10.0%.

Weather analysis. Meteorological data during the study years (2016–2018) were obtained from the database of the Institute of Meteorology and Water Management – National Research Institute (IMGW) from the nearest meteorological station in Zamość. The following parameters were analyzed: average monthly air temperature (°C) and total precipitation (mm) during the amaranth growing season, i.e., from May to October.

Seed germination analysis. The germination capacity of amaranth seeds was determined according to the Polish standard [Polska Norma PN-79R-65950]. Seeds (100) were placed on Petri dishes between two layers of filter paper, soaked in water up to half the height of the seeds, and then incubated at 20 °C for 7 days. Three replicates were performed for each experimental combination. Subsequently, seed germination was assessed, considering only those in which the radicle had penetrated the seed coat.

Seed health analysis. Seed health assessments were conducted based on mycological analysis according to the established methodology for such determinations [Kopacki et al. 2016]. The study used seeds of amaranth varieties 'Aztek' and 'Rawa' collected annually from the field experiment conducted between 2016 and 2018. Sixteen experimental combinations were used for the varieties 'Aztek' and 'Rawa' (NPK0 – control, NPK1, NPK2, NPK3), separately for narrow row and wide row sowing. Seeds (10 seeds per Petri dish × 5 replicates, a total of 50 seeds) from each combination were disinfected for 30 seconds in a 0.1% sodium hypochlorite (NaClO) solution and rinsed three times in sterile water for three minutes each. The seeds were then placed on pre-prepared Petri dishes with a mineral medium of the following composition: sucrose 38 g, NH_4NO_3 0.7 g, $MgSO_4 \times 7H_2O$ 0.3 g, NH_2PO_4 0.3 g, $FeCl_3 \times 7H_2O$ trace, $ZnSO_4 \times 7H_2O$ trace, $CuSO_4 \times 7H_2O$ trace, $MnSO_4 \times 7H_2O$ trace and agar 20 g. The medium was supplemented with distilled water to a volume of 1000 mL and sterilized at 121 °C under a pressure of 1 atmosphere for 20 minutes following the methodology of Kopacki et al. [2016]. Petri dishes with the seeds were placed in an incubator and kept in the dark for 7–10 days at a temperature of 20–22 °C. Fungal colonies that grew from the seed material were then counted and transferred to potato dextrose agar (PDA Difco) slants. The obtained fungal cultures were classified based on macroscopic characteristics, such as the appearance of the mycelium (structure, color), counted, and then identified to the genus or species level using a microscope, available monographs and identification keys [Raper et al. 1949, Booth 1971, Ellis 1977, Sałata and Rudnicka-Jezierska 1979, Nelson et al. 1983, Marcinkowska 2010, Marcinkowska 2012].

Statistical analysis. The data are presented as means from individual study years. The null hypothesis H0 was tested using analysis of variance (ANOVA) with Snedecor's F-test. Additionally, the probability of the F-distribution was calculated. The significance of differences was assessed using Tukey's test (α = 0.05), followed by a post-hoc analysis. Additionally, the coefficient of variation (CV%) was calculated as a measure of result dispersion, defined as the quotient of the standard deviation and the mean and standard error. Correlation and linear regression analyses were conducted to determine the relationships and associations between the studied traits. Statistical analysis

was performed using Excel 7.0 and Statistica (StatSoft Polska, 2013). Excel 7.0 was also used to prepare figures, including pie charts illustrating the percentage distribution of fungi isolated from amaranth seeds.

RESULTS AND DISCUSSION

Weather conditions in the growing seasons of 2016–2018

Weather conditions during the study years were highly variable (Figs 1–2).

In 2016, the sowing of amaranth was carried out in a wet and generally warm May (total precipitation – 49.9 mm, average monthly temperature – 14.3 °C). In the following months of the amaranth growing season, the average air temperature was higher compared to the long-term average. From June to October, the total precipitation in the study area was higher than the long-term average (Fig. 1). In October, a record amount of precipitation was observed (99.9 mm), exceeding the long-term average by over 100%, which significantly hindered the seed harvest.

In 2017, amaranth was sown during a warm spring and rainfall deficit (total precipitation – 52.2 mm, average monthly temperature – 13.7 °C). Temperatures above the long-term average persisted throughout the growing season and were, except for July and August, lower than in the first year of the study. From sowing until October, rainfall was significantly below the long-term average. These meteorological conditions adversely affected the germination, growth, and development of amaranth. September (67.7 mm) and October (66.0 mm) were particularly rainy months, which significantly hindered seed maturation and subsequent harvest.

In 2018, from sowing through the entire growing season until harvest, temperatures were consistently higher than the long-term average. They were also the highest among the studied years (Fig. 2). The precipitation deficit in May (45.2 mm, 37.2% below the

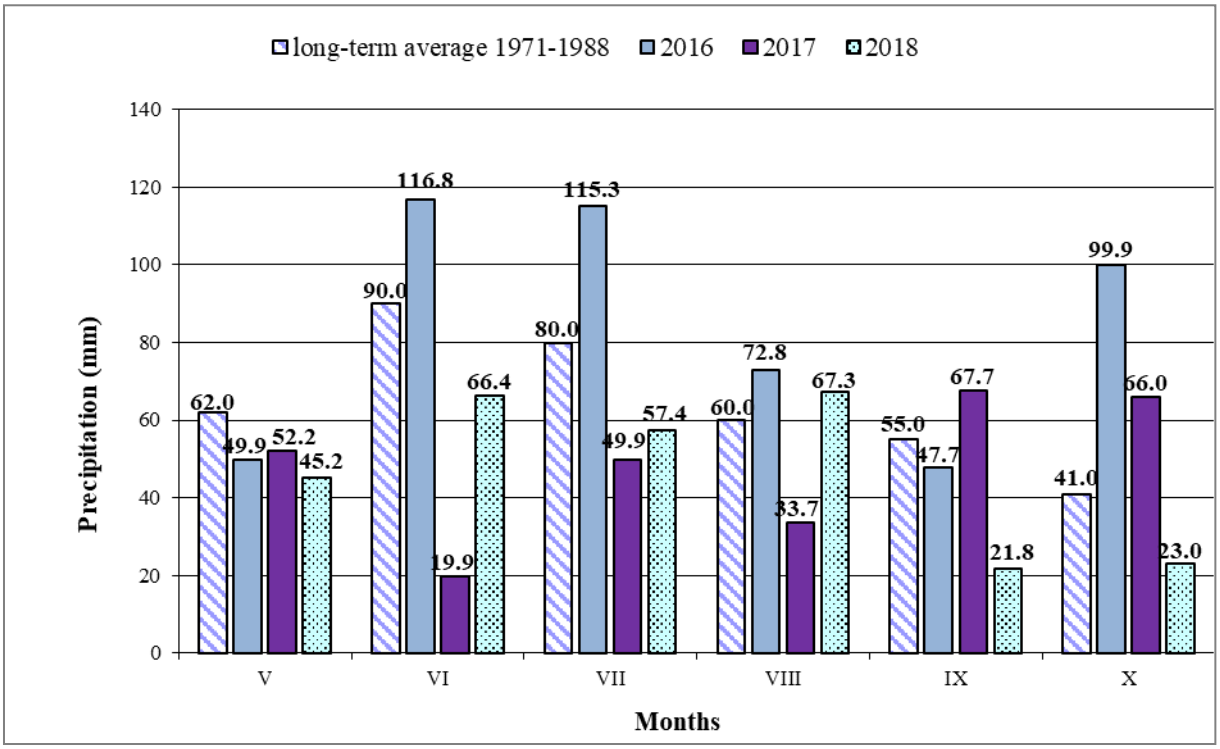

Fig. 1. Precipitation totals (mm) for the years 2016–2018 and the long-term average for 1971–1988 (based on data from the Meteorological Station in Zamość)

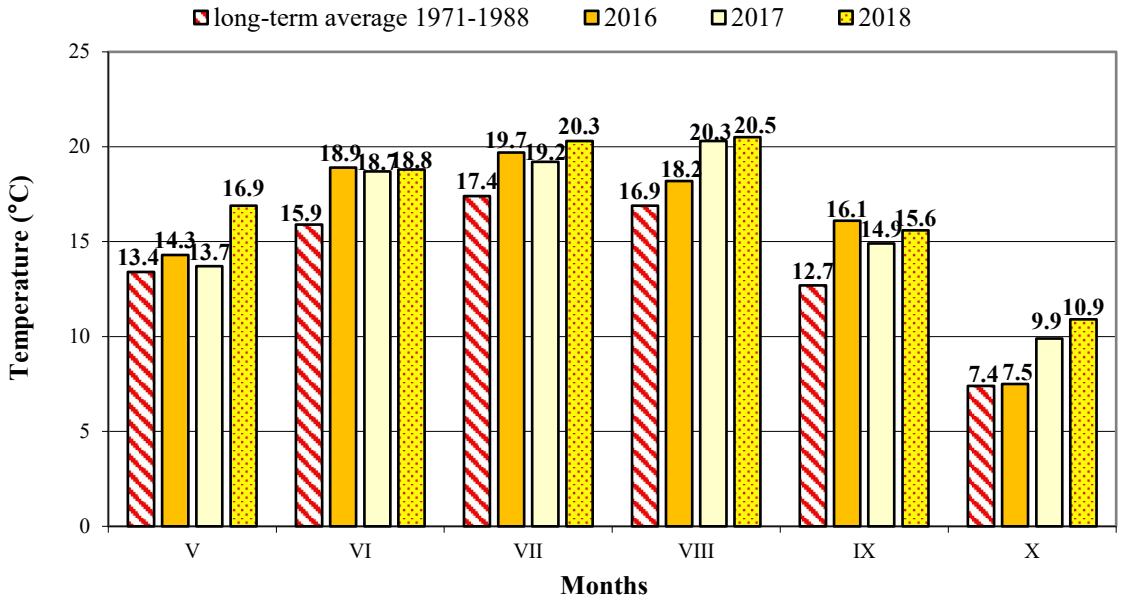

Fig. 2. Average monthly air temperatures (°C) for the years 2016–2018 and the long-term average for 1971–1988 (based on data from the Meteorological Station in Zamość)

long-term average) was the highest among the three years of the experiment. In July, precipitation was also below the long-term average but higher than in 2017. Additionally, the average monthly temperatures in July and August were higher compared to the long-term average. In August, the total precipitation was 7.3 mm higher than the long-term average. Low precipitation in October provided favorable hydrothermal conditions for harvest.

Germination capacity

A synthesis of the three-year study results demonstrated that the germination capacity of amaranth seeds was significantly dependent only on the variety and showed low variability at a level of 19.9% (Tab. 1). The variety 'Aztek' exhibited a higher germination capacity than the variety 'Rawa', with an average difference of 22.5% between the two varieties.

Fungi colonizing seeds

The overall number of fungal colonies on amaranth seeds was significantly affected only by the variety and row spacing, and this parameter exhibited high variability (Tab. 1). The variety 'Rawa' was 2.3 times more colonized by fungi compared to 'Aztek'. Various scientific studies often emphasize that the selection of the appropriate variety is an important factor in successful agricultural cultivation, including seed yield and its health state. Given the significantly limited options of protecting amaranth under the soil and climatic conditions of Poland – primarily due to the lack of recommended plant protection products – the resistance of varieties in production is of great importance [Matyjaszczyk 2011, Krasowska 2022]. For years, especially in countries with hot climates, there has been a search for amaranth species and varieties with increased resistance, particularly to the most dangerous pathogens, such as *Fusarium oxysporum* [Chen and Swart 2001, Chen and Swart 2002]. The current study indicates that, under the soil conditions of south-eastern Poland, the variety 'Aztek' exhibited higher overall seed health compared to the variety 'Rawa'. The overall abundance of fungal colonies on amaranth seeds also depended on row spacing (Tab. 1). Cultivating amaranth in narrow rows led to approximately 1.9-fold higher fungal colonization of the seeds compared to wide row spacing.

The literature often emphasizes that sowing density also affects the success of the cultivation. Excessive plant density is detrimental due to increased competition for light and nutrients, leading to spindly and overgrown plants that are more susceptible to patho-

Table 1. Germination capacity of amaranth seeds and total abundance of *Alternaria alternata* and *Aspergillus brasiliensis* colonies present on the seeds (averages for years and factors)

Factor	Germination capacity (%)	Total colony number of fungi present on the seeds	Number of *Alternaria alternata* colonies on seeds	Number of *Aspergillus brasiliensis* colonies on seeds
2016	75.6 a	47.9 a	22.3 a	5.3 a
2017	72.4 a	56.4 a	14.3 a	6.2 a
2018	68.5 a	56.4 a	13.6 a	8.2 a
$NIR_{0.05}$	12.4	30.8	9.4	5.4
F^0 value	0.9	0.3	3.1	0.9
P value	0.3940	0.7429	0.0550	0.4257
NPK0	67.5 a	39.3 a	8.1 b	4.5 a
NPK1	74.4 a	60.8 a	21.8 a	9.0 a
NPK2	72.1 a	61.9 a	18.6 ab	6.3 a
NPK3	74.6 a	52.4 a	18.3 ab	6.5 a
$NIR_{0.05}$	16.0	38.5	11.4	6.9
F^0 value	0.6	1.0	3.9	1.0
P value	0.6143	0.3826	0.0149	0.3842
W	69.1 a	70.0 a	19.5 a	9.4 a
S	75.2 a	37.2 b	14.0 a	3.7 b
$NIR_{0.05}$	8.3	18.3	6.5	3.3
F^0 value	14.3	13.0	2.9	12.2
P value	2.1349	0.0008	0.0949	0.0011
A	83.4 a	32.3 b	11.4 b	3.3 b
R	60.9 b	74.9 a	22.0 a	9.9 a
$NIR_{0.05}$	5.3	16.5	5.9	3.1
F^0 value	74.2	27.2	12.9	18.1
P value	3.72×10^{-11}	4.31×10^{-6}	0.0077	0.0001
CV (%)	19.9	65.3	67.5	95.0

Legend: NPK0 – control, NPK1 – first fertilization variant, NPK2 – second fertilization variant, NPK3 – third fertilization variant; W – narrow row spacing, S – wide row spacing; A – variety 'Aztek', R – variety 'Rawa'; CV – coefficient of variation

gens. Additionally, increased humidity in a dense stand promotes fungal spore germination and disease development [Próchniak 2005, Krasowska 2022]. The conducted experiments demonstrated that the number of fungi colonizing amaranth seeds was influenced solely by the interaction between row spacing and variety. Wider row spacing contributed to better seed health status compared to narrower row spacing, with this effect being more pronounced for the variety 'Aztek' than for 'Rawa'.

The germination capacity of amaranth seeds was significantly negatively correlated with the overall abundance of fungal colonies present on the seeds ($r = –0.6564$) – Fig. 3. Similar results were obtained by Irzykowska and Kononowicz [2017] in their study on sunflower seeds. The latter authors demonstrated that increased seed colonization by fungi from the genera *Fusarium, Penicillium, Mucor*, and *Alternaria* significantly decreased seed germination capacity.

A detailed mycological analysis during the study years revealed both quantitative and qualitative variation in fungi colonizing the amaranth seeds (Tabs 2 and 3). Saprotrophic fungi, as well as pathogenic fungi, primarily of the genus *Fusarium*, were predominantly identified on the seed material under study. Particular attention was paid to pathogenic fungi that produce mycotoxins, which contaminate the seeds. The obtained isolates included species considered antagonists to pathogens, which are increasingly being used with greater effectiveness in biological plant protection.

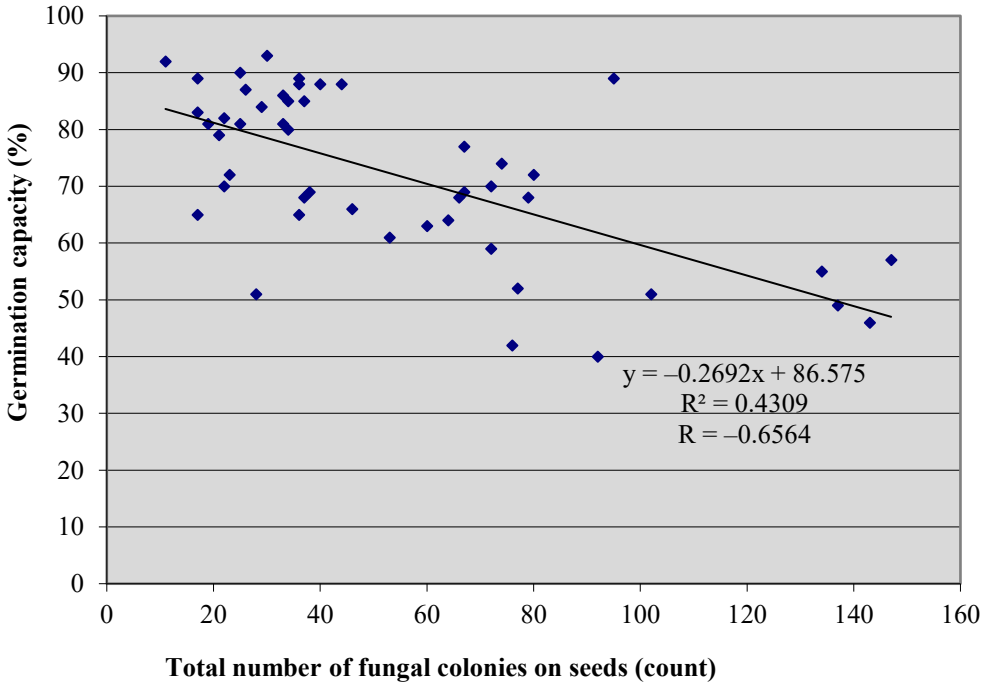

Fig. 3. Relationship between seed germination and the total number of fungal colonies present on the seeds

During the study period, a total of 765 fungal colonies of 15 species were isolated from the seeds of the variety 'Aztek', while 1798 colonies of various species were isolated from the variety 'Rawa' (Table 2, 3). The dominant fungi colonizing the seeds identified in the study were *Alternaria* sp. and *Aspergillus brasiliensis*, as well as fungi from the genera *Penicillium* and *Fusarium* (Tab. 2, 3). The dominant species on the variety 'Aztek' was *Alternaria* sp. (Tab. 2). Its presence was observed in nearly all experimental combinations from 2016 to 2018, except for NPK1 plots with wide row spacing in 2018. This fungus colonized the seeds from all analyzed combinations, most often those remaining from NPK1 plots, regardless of row spacing, and in NPK2 from narrow row cultivation (Tab. 2). The species *Aspergillus brasiliensis* was identified in every cultivation system each year, regardless of fertilization rate or row spacing, with particularly high abundance in the third year of the study (Tab. 2). Fungi *Fusarium* sp. was found sporadically, with particularly high abundance in the third year of the study on seeds from the control and NPK2 treatments with wide row spacing (Tab. 2).

Seeds of the variety 'Rawa' were predominantly colonized by *Alternaria* sp. (49.0% in 2016, 21.0% in 2017, and 23.0% in 2018). This species inhabited seeds from all combinations, both wide row and narrow row plantations (Tab. 3). *Aspergillus brasiliensis* was present every year and accounted for 13.0%, 11.0%, and 15.0% of all isolates during the successive study years, respectively. It occurred particularly on seeds from narrow row spacing plots (Tab. 3). *Fusarium* sp. were isolated less frequently and primarily colonized seeds from narrow row spacing plots (Tab. 3). It should be noted that a dangerous strain of *Botrytis cinerea* was isolated in 2018, particularly from seeds derived from narrow row cultivation, accounting for 5.0% of the isolates (Tab. 3). The number of *Alternaria* sp. colonies on amaranth seeds significantly depended on the variety and fertilization dose (Tab. 1). Seeds of the variety 'Rawa' were almost twice as likely to be colonized by this fungus compared to the variety 'Aztek' (Tab. 1).

Alternaria sp. was the most frequently isolated genus from amaranth seeds of both varieties. This was consistent with many previous studies focusing

Table 2. Fungi colonizing cultivar 'Aztek' in the two sowing variants

Year	Fungus species	Wide row spacing				Narrow row spacing				Total (%)
		NPK0	NPK1	NPK2	NPK3	NPK0	NPK1	NPK2	NPK3	
2016	*Alternaria* sp.	6	26	8	16	11	20	14	2	103(47)
	Aspergillus brasiliensis	2	–	–	4	4	–	–	5	15(7)
	Chaetomium cochlioides	–	–	–	–	2	7	–	–	9(4)
	Epicoccum nigrum	4	–	2	–	5	–	–	–	11(5)
	Fusarium sp.	2	–	–	2	2	–	2	–	8(4)
	Penicilium chrysogenum	3	2	–	–	3	–	–	–	8(4)
	Penicilium expansum	2	3	–	–	4	–	–	–	9(4)
	Rhizopus stolonifer	2	–	–	–	2	–	6	5	15(7)
	Stemphylium botryosum	–	3	7	–	–	4	4	11	29(13)
	Trichoderma koningii	4	2	–	–	4	2	–	–	12(5)
	Total	25	36	17	22	37	33	26	23	219(100)
2017	*Alternaria* sp.	4	14	2	7	9	21	19	11	87(38)
	Aspergillus brasiliensis	2	–	2	3	4	7	–	4	22(9)
	Cladosporium cladosporoides	–	3	–	–	–	–	2	–	5(2)
	Epicoccum nigrum	2	–	4	2	6	–	2	–	16(7)
	Fusarium sp.	–	–	2	–	2	–	3	–	7(3)
	Penicilium chrysogenum	–	–	–	2	–	–	–	–	2(1)
	Penicillium spinulosum Link	2	–	2	–	4	4	2	–	14(6)
	Rhizopus stolonifer	2	4	4	3	3	2	4	3	25(11)
	Trichoderma harzianum Rifai	6	–	–	–	8	–	3	–	17(7)
	Trichoderma koningii Oud.	3	4	3	–	2	6	2	11	31(13)
	Total	21	25	19	17	38	40	37	29	226
2018	*Alternaria* sp.	7	–	13	5	6	15	21	17	84(26)
	Aspergillus brasiliensis	2	3	2	2	3	8	12	9	41(13)
	Cladosporium cladosporioides	–	3	7	–	–	2	3	–	15(4)
	Epicoccum nigrum Link	4	–	–	–	4	5	4	–	17(5)
	Fusarium sp.	4	9	–	4	3	–	17	–	37(11)
	Penicillium chrysogenum	–	2	5	–	–	–	8	6	21(6)
	Penicilium expansum	3	–	–	–	3	7	4	–	17(5)
	Penicilium spinulosum	3	–	7	–	4		–	2	16(5)
	Rhizopus stolonifer	3	5	–	–	2	7	5	–	22(7)
	Trichoderma harzianum	5	–	–	–	5	–	9	2	21(6)
	Trichoderma koningii Oud.	5	8	–	–	4	–	12	–	29(9)
	Total	36	30	34	11	34	44	95	36	320

on yield contaminations [Blodgett and Swart 2002, Pusz 2008, Pusz 2009a, Pusz et al. 2015]. According to Noelting et al. [2016], the frequent occurrence of *Alternaria alternata* on sterilized seed surfaces could result from the direct colonization of flowers and seeds by this fungus during flowering. Narkiewicz-Jodko [1986, 1998] and Narkiewicz-Jodko and Gil [1997] reported that the presence of *Alternaria alternata* on seeds did not negatively affect germination capacity. However, the present study indicated reduced germination capacity and increased fungal infection of seeds of the variety 'Rawa', especially those grown in narrow row spacing (Tab. 1) – Fig. 4.

Fungi of the genus *Alternaria* are frequently identified as the primary cause of seed [Noelting et al. 2016] and leaf [Blodgett and Swart 2002] contamination of many amaranth species. In addition, they are characterized by high genetic variability and easily develop races resistant to different groups of fungicides [He et al. 2019, Pusz 2009a, Saharan et al. 2016]. Therefore, alternative methods, such as biological protection, are being sought to eliminate these fungi from seeds [Aslam et al. 2010, Jensen et al. 2004]. The number of *Alternaria* sp. colonies on amaranth seeds was significantly affected by the level of NPK fertilization. More colonies of this pathogen were found on seeds

Table 3. Fungi colonizing cultivar 'Rawa' in the two sowing variants

Year	Fungus species	Wide row spacing				Narrow row spacing				Total (%)
		NPK0	NPK1	NPK2	NPK3	NPK0	NPK1	NPK2	NPK3	
2016	*Alternaria* sp.	6	40	45	42	15	34	36	45	263(49)
	Aspergillus brasiliensis	2	10	16	–	11	18	5	8	70(13)
	Chaetomium cochlioides	–	–	–	–	4	–	5	5	14(2)
	Epicoccum nigrum	2	–	3	8	6	9	4	5	37(7)
	Fusarium sp.	4	3	4	6	13	14	4	1	49(9)
	Penicilium expansum	3	–	–	2	6	–	2	6	19(3)
	Penicilium spinulosum	2	–	–	7	6	–	–	3	18(3)
	Rhizopus stolonifer	–	8	6	–	2	–	15	–	31(5)
	Trichoderma harzianum	2	5	–	–	5	–	–	–	12(2)
	Trichoderma koningii	2	–	–	2	4	5	6	6	25(4)
	Total	23	66	74	67	72	80	77	79	538
2017	*Alternaria* sp.	4	11	13	17	13	31	27	25	141(21)
	Aspergillus brasiliensis	2	6	9	2	6	21	13	18	77(11)
	Cladosporium cladosporoides	–	–	–	5	2	–	21	6	34(5)
	Epicoccum nigrum	2	9	–	–	2	4	–	–	17(2)
	Fusarium sp.	5	16	16	13	10	27	34	54	175(25)
	Penicilium chrysogenum	–	–	13	–	2	21	2	–	38(6)
	Penicilium expansum	–	6	–	–	3	–	15	2	26(4)
	Penicillium spinulosum	3	5	–	–	5	12	–	–	25(3)
	Rhizopus stolonifer	–	3	3	–	–	9	3	–	18(2)
	Stemphylium botryosum	–	–	–	–	2	–	3	6	11(1)
	Trichoderma harzianum Rifai	–	3	5	7	3	11	4	13	46(6)
	Trichoderma koningii Oud.	6	5	8	2	12	11	15	10	69(10)
	Total	22	64	67	46	60	147	137	134	677
2018	*Alternaria* sp.	5	22	3	19	11	28	22	24	134(23)
	Aspergillus brasiliensis	4	12	–	4	12	23	16	19	90(15)
	Botrytis cinerea	–	–	3	4	4	11	2	9	33(5)
	Fusarium sp.	2	11	4	6	6	7	47	15	98(16)
	Penicillium chrysogenum	–	–	2	–	–	–	14	11	27(4)
	Penicilium expansum	5	9	–	–	11	–	–	7	32(5)
	Penicilium spinulosum	3	6	–	–	7	9	9	–	34(6)
	Phoma herbarum	–	–	4	–	–	–	–	–	4(1)
	Rhizopus stolonifer	2	–	–	7	6	5	11	9	40(7)
	Trichoderma harzianum	5	–	1	2	12		9	–	29(5)
	Trichoderma koningii Oud.	2	12	–	11	7	9	13	8	62(11)
	Total	28	72	17	53	76	92	143	102	583

obtained from the plots with the lowest fertilization level (NPK1) compared to the control (NPK0). Literature reports often highlight the significant impact of fertilization on pathogen development, particularly after the introduction of high nitrogen doses [Amanullah et al. 2014]. Leaves and shoots grow excessively and become more susceptible to infection [Masternak and Kulikowska 2010, Krasowska 2022].

In the current study, the number of *Aspergillus brasiliensis* isolates from amaranth seeds significantly depended on the variety and row spacing (Tab. 1) – Fig. 5. Seeds of the variety 'Rawa' were three times more frequently colonized by *Aspergillus brasiliensis* compared to the variety 'Aztek'. Narrow row sowing also favored seed colonization by this fungus compared to wide row cultivation (Tab. 1).

The current study also found an effect of the interaction between row spacing and varietal factor on the number of *Aspergillus brasiliensis* isolates from the surface of amaranth seeds.

Previous studies confirm the frequent occurrence of *A. brasiliensis* and other fungal species causing

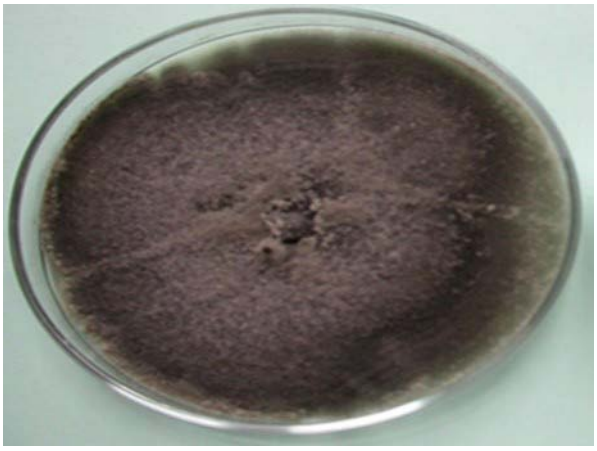

Fig. 4. Ten-day-old colony of *Alternaria alternata* isolated from amaranth seeds of the variety 'Rawa' in 2016 (photo by P. Krasowska)

Fig. 5. Seven-day culture of *Aspergillus brasiliensis* isolated from amaranth seeds of the variety 'Aztek' in 2017 (photo by P. Krasowska)

contamination of amaranth seeds and leaves, affecting yield purity and germination [Hamim et al. 2014, Akinnibosun and Adeola 2015]. These species are present in the soil, particularly in the root zone of plants, but are also found on seeds, as confirmed by the present study. Considering the rapid spore production, storage conditions of amaranth seeds and the application of all available protection methods are crucial [Ninganagouda et al. 2014, Yassin et al. 2016].

The hydrothermal conditions during the study years were one of the primary factors determining the abundance of fungi colonizing seeds. The present study found no significant effect of weather conditions on overall fungal abundance, including the colonization of amaranth seeds by *Alternaria* sp. and *Aspergillus brasiliensis*. However, it should be noted that the highest rainfall was recorded in 2016, followed by 2018, and the lowest precipitation was recorded in 2017 (Fig. 1). The highest percentage of fungi from the genus *Alternaria* was observed in 2016, while *Aspergillus* species were predominantly responsible for the contamination of amaranth seeds in 2018. It should also be noted that precipitation plays a significant role in the colonization of plants during the growing season, as well as seeds, by pathogenic and saprotrophic fungi. This may also be related to the very high water absorption by the inflorescences and fruits of amaranth [Szot 1999]. Excessive rainfall affects the high seed moisture during the harvest period (third decade of September to October). Therefore, rapid drying of seeds after harvest is crucial, as the moisture and temperature of the stored material impact the survival of pests and, consequently, affects seed quality [Nunuparov et al. 2019]. Fungi of the genus *Fusarium*, especially *A. cruentu*s and *A. retroflexus*, are often isolated on amaranth seeds [Pusz et al. 2015]. This was also confirmed in the present study: *Fusarium equiseti* was isolated from 'Aztek' seeds in 2016 and 2018, and from 'Rawa' seeds from 2016 to 2018, *F. avenaceum* from 'Aztek' seeds in 2017 and from 'Rawa' seeds from 2016 to 2018, *F. oxysporum* from 'Aztek' seeds in 2016 and 2018, and from 'Rawa' seeds in 2016 and 2017, *F. culmorum* was exclusively isolated from 'Rawa' seeds in 2016 and 2018 (Tabs 2 and 3).

The presence of *Fusarium* spp. on seeds indicates their lower quality, associated with mycotoxin production [Sadowski et al. 2007, Hassani et al. 2019, Mielniczuk and Skwaryło-Bednarz 2020]. Allemann and Denner [2006] reported that the low frequency of fungi other than *Fusarium* spp. could be due to the fact that amaranth is known as an allelopathic plant, exhibiting the ability to inhibit the development of plant pathogens.

The sporadically isolated species *Chaetomium globosum*, which belongs to antagonistic fungi, is also utilized in biological control [Fierro-Cruz et al. 2017]. However, some researchers have not observed an antagonistic effect of this fungus against *Aspergillus* sp. or *Penicillium* sp. [Fojutowski and Kropacz 2015].

Given that pathogens affecting yields can be transmitted through both soil and seeds, it may be necessary in the future to consider implementing alternative legal seed treatment methods as one of the primary means of protection [Lamichhane et al. 2020]. Plant health improvement can also be achieved through the application of mycorrhizal vaccines [Jamiołkowska et al. 2020]. In order to remove pathogens or pests from grains, fumigation with pesticides or safer physical methods are increasingly used [Chen et al. 2020, Sankar et al. 2020, Kopacki et al. 2021]. Additionally, biological methods employing *Epicoccum nigrum*, which was also isolated in the current study (every year from 'Aztek' seeds and in 2016–2017 from 'Rawa' seeds), are utilized [Jensen et al. 2016].

CONCLUSIONS

The results obtained from the field experiment led to the following conclusions.

The narrow row cultivation of amaranth and the application of high doses of NPK fertilizers should be limited, as they contribute to plant crowding and seed colonization by various fungal species, particularly in the 'Rawa' variety. The highest number of pathogen colonies was obtained from seeds of plants cultivated in narrow row spacing in combination with the application of the lowest fertilization level. Dominant mycotoxin-producing species included *Alternaria* sp. and *Aspergillus brasiliensis*.

In the soil and climatic conditions of the Zamość region, it is recommended to grow the 'Aztec' variety in wide row spacing, using the second fertilization option NPK2 (110 N, 70 P_2O_5, 70 K_2O). This approach is considered a rational mineral fertilization practice for amaranth, aligning with the principles of integrated production systems adopted by Polish agriculture. Such practices aim to apply mineral fertilization below the level of uptake, which also promotes good post-harvest seed health.

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