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Evaluation of biofumigation plants on *Fusarium* crown rot and head blight of wheat in North Sinai

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ABSTRACT

Fusarium head blight and Fusarium crown rot diseases caused by *Fusarium sp.* are among the most severe plant diseases in the world. diseased samples were collected from fields in Al-Arish in late May 2021 and 2022. In this study an isolate of *Fusarium nygamai* was identified with a GenBank accession number OQ152508. The pathogenicity tests on six cultivars of wheat (Misr1, Misr3, Giza171, Sids14, Egaseed22, and Sakha 95) showed that cultivar Giza171 had the highest percentage of disease severity (3.33). In contrast, cultivars Sids14 and Saka95 had the least percentage of disease severity (1.25). Disease incidence of Misr1 and Misr3 cultivars had the maximum rate at (100, and 100 %) respectively. This study aimed to explore biofumigation as a sustainable alternative to conventional fungicides by assessing the impact of four biofumigant plants (Cabbage, Turnip, Rocket, and Radish) on the pathological characteristics of five wheat cultivars (Misr1, Misr3, Giza171, Sakha95, and Sids14), in comparison to a control treatment for controlling crown rot and head blight of wheat. In vitro, biofumigation with rocket treatment was the most effective treatment that reduced the mycelial growth of *Fusarium nygamai*. Greenhouse, biofumigation with rocket was the most effective treatment that reduced severity and incidence of disease with *F. nygamai* in both seasons. In addition, it decreased the infected spike number with isolate *F. nygamai*. Laboratory and Greenhouse trials proved that biofumigation was effective in controlling FCR disease of wheat as an environmentally friendly control treatment.

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Introduction

Wheat is the most important grain crop all over the world. Wheat is cultivated as a cash crop, as it produces a good yield per unit area, grows well in a temperate climate with a moderately short growing season, and yields versatile, high-quality flour. Most wheat flour is used to make products including bread, pasta, cereals, pastries, cookies, crackers, muffins, tortillas, and pizza. In 2022 wheat production in Egypt amounted to approximately 9.3 million metric tons. However, in 2023 it recorded 8.7 million metric tons which showed a decrease of 0.6 million metric from the preceding year. Egypt needs to import

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about 11 million metric tons of wheat and wheat-derived products in 2023/24.

More than one-third of the world's population relies on wheat as an essential food, making it the major grain crop. To reduce the difference between the achievable and actual yields, it is essential to understand illnesses that may cause damage and are likely to impair plant health and quality. Diseases are a key factor in crop loss for wheat. Increasing wheat yield potential in developing countries remains a top concern due to the growing global population and the expected growing importance of food security (Gaunt, 1995). Epidemics are caused by the interaction of the inoculum, a conducive environment, and the host's vulnerability. Therefore, minimizing infections and the difference between actual and attainable yields depends on understanding pathogens, their ecology, distribution, virulence patterns, and variability (Wiese, 1987).

Wheat infections result in harvest and storage losses and poor harvest crop quality. The degree and diversity of the pathogens'inoculum, the environmental factors, and the genetically determined resistance and tolerance of the wheat cultivars to particular diseases may all affect yield losses. Fusarium diseases cause major yield and quality losses on many economically important plant species including cereals. Fusarium crown rot (FCR) is one of the most dangerous diseases of wheat (*Triticum aestivum* L.) in Egypt. Many fungal pathogens have been recorded as responsible for causing this disease. in Egypt, *Fusarium nygamai* recorded the first time from rhizoplane of lentil (Abdel-Hafez et al .2012),isolated from potato (Abo-Elnaga et al. 2013) and succeeded in isolating it from sorghum and lentil (Abdel-Hafez et al . 2014).

Fusarium nygamai has been previously reported and recovered from wheat root and stalk (Fard et al. 2017) and caused root rot on wheat in Iraq (Minati, 2020), rice in Sardinia (Balmas et al. 2000), sugar beet in China (Cao et al. 2018), lentil in Pakistan (Rauf et al. 2016) as well as Crown rot of wheat in China.

FHB is another important wheat disease that causes major agricultural problems worldwide. Fusarium head blight (FHB) in wheat is primarily caused by several Fusarium species, including F. *pseudograminearum*, *F. avenaceum*, *F. culmorum*, and *F. graminearum* (Smiley et al., (2005); Ali & Mahmoud (2019). While *F. graminearum* is the dominant FHB pathogen in North America, the specific species causing FHB can vary regionally (Goswami & Kistler,2004).

In Egypt, for example*, F. graminearum* was identified as the primary culprit (Mahmoud, 2016), while in Canada, it's just one of several Fusarium species contributing to FHB (Dexter et al.,1997). However, Li et al. (2010) suggested that Fusarium head blight and crown rot are two wheat diseases caused by the same Fusarium pathogens. This study was undertaken to identify and provide descriptions of fungal pathogens linked to crown rot in wheat disease using morphological and cultural characteristics and to determine the pathogenicity of fungi causing crown rot, specifically *Fusarium nygamai* in North Sinai. and to test the efficiency of biofumigation with five biofumigant species on growth and pathological characteristics of five wheat cultivars infected with *Fusarium nygamai* under invitro and greenhouse conditions in North Sinai, Egypt.

Materials and Methods

Sampling

On April 25, 2022, diseased wheat plant samples were collected at the ripening stage from the farm of the Faculty of Environmental and Agricultural Sciences at Arish University (Fig 1 a, b). Eight samples were collected from eight wheat cultivars, Misr1, Misr3, Sakha 94, Sakha95, Sids 14, Giza 168, Giza 171, and Sids 12.

The plants were placed individually in air-tight plastic collection bags for transporting to the laboratory to avoid damage to wheat roots and stored at 4°C. The roots were, carefully washed with tap water for 20 min, and cut into small particular parts (1cm), Plant tissues were disinfested 2% two minutes in sodium hypochlorite, three rinses in sterile distilled water, and then air dried on filter papers. They were placed in PDA petri plates (9cm) in three duplicates, with three disinfected root portions per petri dish. Petri plates were incubated at 25°C for seven days and monitored daily for fungal growth. All fungus that grew on these dishes were examined. Only isolates with a Fusarium-like appearance were sub-cultured onto (PDA) with three replicates for each, incubated under comparable conditions, and identified visually and molecularly to genus and species levels.

Morphological characterization of Fusarium isolate

The appearance of colonies, including the rate of mycelium growth, colony characteristics, and color pigmentation, was evaluated on Potato Dextrose Agar (PDA) medium under a microscope at a temperature of 25°C. according to Toussoun et al. (1983). For the investigation of the morphological characteristics of *Fusarium* isolates, uncontaminated samples were cultivated on Potato Dextrose Agar (PDA) dishes and species identification was carried out seven days after incubation at a temperature of 25°C, following the procedures described by Leslie and Summerell (2006).

Molecular identification of the fungal isolate

Fungal cultures were incubated on potato sucrose agar for 5 days at 28°C., following the protocol outlined by (Pitt & Hocking 2009). DNA extraction from fungal samples

was performed at the Molecular Biology Research Unit, Assiut University, using the Patho-gene-spin DNA/RNA extraction kit from Intron Biotechnology Company (Korea). Subsequent amplification of the ITS region of the rRNA gene was carried out using universal primers (ITS1 and ITS4) at SolGent Company (Daejeon, South Korea), with the specific compositions: ITS1 (5' - TCCGTAGGTGAACCTGCGG - 3') and ITS4 (5'- TCCTCCGCTTATTGATATGC -3'). Sequencing utilizing the same primers as the initial PCR, a sequencing reaction was performed on the purified PCR product with the incorporation of ddNTPs in the reaction mix, following the protocol outlined by White et al. (1990). The generated sequences were subjected to similarity searches using BLAST on the NCBI website. Subsequently, MegAlign software version 5.05 (DNA Star) was employed for detailed sequence analysis and the construction of phylogenetic trees to visualize the evolutionary connections between these sequences.

Pathogenicity Test

This experiment aimed to assess the pathogenicity of *Fusarium nygamai* on six cultivars of wheat. Wheat seeds were sown in pots with a diameter of 20 cm, each pot was filled with a soil mixture composed of peat moss and sand, mixed in a volume ratio of one part peat moss to two parts sand. To establish the pathogenicity of seed germination and disease severity, *Fusarium nygamai* inoculum was prepared by placing a 1 cm disc of the isolated fungi into 250 ml conical flasks containing 100 gm of autoclaved barley. The flasks were incubated at 28±2°C for 3-4 weeks, with periodic shaking for 5 minutes over 5 days to ensure uniform distribution of fungal growth. Subsequently, 25 grams of *Fusarium nygamai*-inoculated barley grain medium were mixed with the soil mixture. The wheat seeds were disinfected using 70% ethanol for 60 seconds, 2% sodium hypochlorite for 120 seconds, rinsed three times in distilled water, and dehydrated using filter papers. Planting took place on November 22, 2022 at a rate of five seeds per pot, with four replicates for each cultivar. The pots were situated in a greenhouse. Following seedling development, plants were reduced to three per pot and fertilized every two weeks with a 20-20-20 (N-P-K) solution concentrated at 1%, starting five weeks after sowing. Disease severity: FCR severity was determined by using a crown rot rating (CRR) scale of 1 to 4 for the first internode of each plant, where: 1 = 0–25%; 2 = 25–50%; 3 = 50–75%; and 4 = 75–100% of the internode discolored as described by Hogg et al. (2007). and Disease incidence = (number of infected plants/total number of plants) × 100 according to (Moya, 2010) and Akinsanmi et al.,2004) and the number of seed germination was observed and recorded every day. The standard to determine seed germination rate (%) = 7d number of germinated seeds / total seed number for testing ×100 (Jiang et al., 2014). A randomized complete block design (RCBD) was used for the experiment.

$$\text{Disease incidence} = \frac{(\text{number of infected plants})}{(\text{Total number of plants})} \times 100$$

$$\text{Seed germination rate (\%)} = \frac{(\text{7d number of germinated seeds})}{(\text{Total seed number for testing})} \times 100$$

Greenhouse experiment

Biofumigation control plant materials

In this study, four biofumigant plants were employed, namely cabbage (*Brassica oleracea var. oleracea*), turnip (*Brassica rapa var. rapa*), rocket (*Eruca sativa*), and radish (*Raphanus raphanistrum subsp. sativus*). For the greenhouse experiment, five specific cultivars were selected, namely Misr1, Misr3, Giza171, Sakha95, and Sids14. Seeds for these cultivars were sourced from the Agriculture Research Centre in Giza, Egypt.

Soil infestation

Plastic pots with a diameter of 20 cm were utilized, each containing 2 kg of autoclaved sandy soil. Soil infestation took place one week after the incorporation of Brassica crops. The inoculum preparation involved adding a 1 cm disc of the tested fungi *Fusarium nygamai* and 250 ml flasks containing 100 gm of autoclaved barley. Subsequently, the pots were filled with autoclaved soil and infested with each tested fungal inoculum at a rate of 5% of the soil weight. Wheat seeds were then planted in the inoculated soil 24 hours after the incorporation of Brassica crops.

Preparing for greenhouse experiments

Four Biofumigant species: cabbage, turnip, rocket, and radish were investigated as potential soil biofumigants against fungal pathogens. Both biofumigants were tested in sterilized soil. Four pots each for cabbage, turnip, rocket, and radish were planted with seeds on September 3rd and 5th in two separate seasons.

Standard agricultural practices were followed throughout the growing period. At maturity (December 9th), all biofumigant plants were incorporated back into their respective pots by chopping and mixing them into the soil. The soil was then irrigated to field capacity. To trap the gases released during biodegradation, each pot was covered with a transparent plastic film for 21 days. Pathological characteristics were measured:

Infected spike percentage: Infected spike number / total spike number× 100

Disease incidence: Infected plants / Total plants number ×100

Disease severity

FCR severity was determined by using a crown rot rating (CRR) scale of 1 to 4 for the first internode of each plant, where: 1 = 0–25%; 2 = 25–50%; 3 = 50–75%; and 4 = 75–100% of the internode discolored as described by Hogg et al. (2007).

Laboratory experiments

Evaluate the impact of different biofumigant plants on the mycelial growth of fungal pathogens

An experiment was undertaken to evaluate the impact of different biofumigant plants cabbage, Turnip, Rocket and Radish. on mycelial growth of the isolated fungal pathogens. Petri plates (90 mm diameter) containing 15 ml PDA were inoculated centrally with a 5 mm disk cut from an actively growing fungal growth margin. The experiment aimed to assess the growth inhibition of the pathogens by volatile biocidal compounds from macerated biofumigant tissues.

At flowering stage, biofumigant tissues were collected, washed, disinfected (ethanol 70% for ten seconds), rinsed in SDW for 5 minutes, dried, and macerated. Aseptically, 7g of macerated tissues from each species were placed in Petri dish lids (Fan et al., 2008)

The experiment was conducted for two seasons tracking the growth of two distinct fungal isolate *F, nygamai*. over time. For each isolate, colony diameter was measured in two perpendicular directions at three specific time intervals: 3-, 5-, and 7-days post-inoculation. The percent reduction in radial growth for each treatment compared to the control was then calculated 7 days after inoculation using the formula described by Fan et al. (2008).

$$\text{Percent growth inhibition(PGI)} = \frac{\text{Colony growth in control plate) - (Colony growth in intersection plate)}}{\text{(Colony growth in control plate)}} \times 100$$

Design and statical analysis

All data were analyzed using SPSS software (v16.0). Two-way ANOVA Mean ± SD (n = 3) tested main and interaction effects, followed by Duncan's test for significant differences among means ($P \leq 0.05$) (Duncan, 1955). Treatments were replicated four times in each experiment and arranged in a completely randomized design (CRD).

Results

Isolate identification

The phenotypic examination of the studied fungal isolate indicated that the recovered taxon was *Fusarium nygamai* according to Leslie and Summerell (2006). The isolate was characterized by secreting a white pigment on PDA then gradually turned to violet. Under a microscope Single-spored isolates were obtained; macroconidia were fluffy, tender-walled, and measured 7.5µm -13.75µm ×1.5 µm -3.75 µm between 3 and 5 septate, microconidia with 0 - 1 septate measured 5µm - 7.5µm × 1.25 µm – 2.5µm (*scale*=40) after eight days of incubation (Fig. 1 c,d). also, chlamydospores appeared after 11 days of incubation and measured 2.5 µm – 6.25 µm (*scale*=40). Sample was deposited at Assiut University Mycological Center under accession number AUMC 15956.

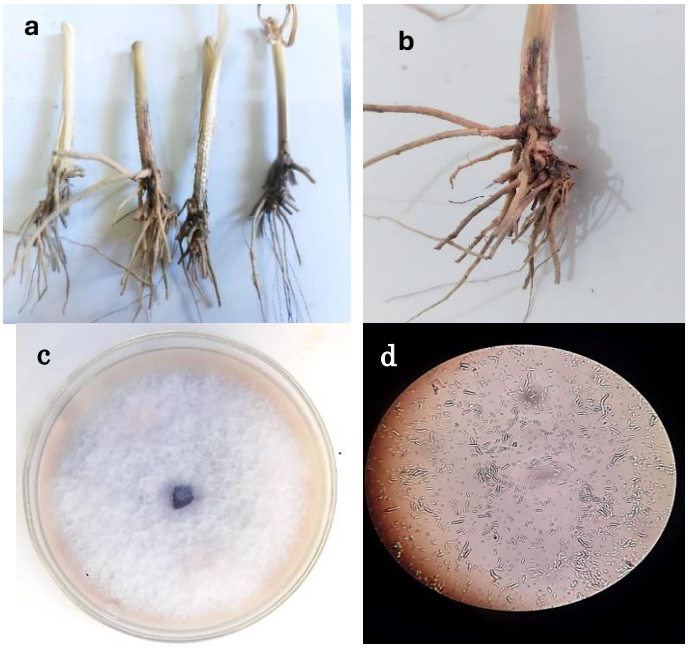

Fig 1. A and B**, s**ymptoms of crown rot disease on wheat, C, morphology of *Fusarium nygamai* on PDA and D, macroconidia and microconidia.

Molecular characterization

A portion of the internal transcribed spacer (ITS) region of the fungal DNA was amplified by polymerase chain reaction (PCR). The resulting sequence was then subjected to a similarity search using the Basic Local Alignment Search Tool (BLAST) on the National Center for Biotechnology Information (NCBI) website. Finally, phylogenetic trees were generated using MegAlign software (DNA Star) version 5.05 to visualize evolutionary relationships among the obtained sequences (Fig. 2). The isolate no S56 was deposited in the GenBank under accession number OQ152508. The current isolate

exhibited a high level of identity ranging from 99.81% to 100%, and coverage ranging from 99% to 100%, with the closest strains, including the type material *F. nygamai* strain NRRL 13448 (GenBank accession no. NR_130698). Additionally, *Fusarium* species in Clade 2 displayed some genetic relationship with *F. nygamai*. The outgroup strain *Sarocladium strictum* is also included for reference. Abbreviations: F. = *Fusarium*, S. = *Sarocladium.*

Pathogenicity test

The infection of selected six wheat cultivars displayed different susceptibility and resistance levels. Data presented in Table (1) showed that Giza 171 cultivar had the highest number of disease severity (3.33), followed by cultivar Misr3, and Misr1 (3.08،3.00) respectively, indicating that they were the most susceptible cultivars compared to others. While the cultivars Sids14 and Sakha95 had the least number of disease severity (1.25 ، 1.25) respectively.

Fusarium nygamai had a significant effect on seed germination rate of all cultivars of wheat examined compared to control as cultivar Giza171 had the least effect of *Fusarium nygamai* on seed germination as record (15.00) but Sakha 95 had the most effect of *Fusarium nygamai* on seed germination compared to control as recorded (60) % respectively.

Disease incidence had the highest percentage with cultivars Misr1 and Misr3 as recorded (100,100) % respectively but other cultivars Giza171, Sids14, Egaseed22, and Sakha95 recorded the least same percentage (91.66) %.

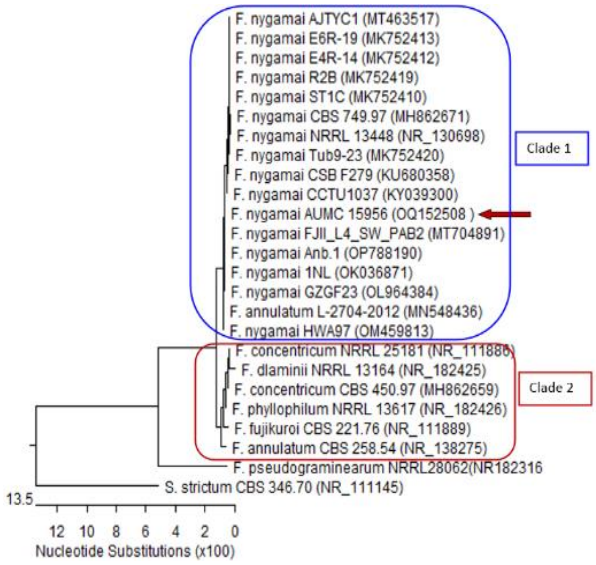

Fig 2. A phylogenetic tree based on ITS sequencing of rDNA illustrates the fungal strain *Fusarium nygamai* isolate S56 (= AUMC15956 with GenBank accession number OQ152508, indicated by an arrow) aligned with closely related sequences from fungal strains obtained from GenBank (Clade 1).

Table 1 Pathogenicity results of *Fusarium nygamai* isolate on wheat cultivars presented seed germination at 7 days after planting and Disease severity and Disease incidence at 90 days after inoculation

		Interaction					
		Misr1	Misr3	Giza171	Sids14	Egaseed22	Sakha95
Disease severity	infected	3.00	3.08	3.33	1.25	2.41	1.25
	Un infected	0.00	0.00	0.00	0.00	0.00	0.00
Disease	infected	100.00	100.00	91.66	91.66	91.66	91.66
incidence	Un infected	0.00	0.00	0.00	0.00	0.00	0.00
seed	Infected	55.00	40.00	15.00	50.00	40.00	60.00
germination rate	Un infected	90.00	50.00	50.00	70.00	85.00	90.00
		**	**	**	**	**	**

Greenhouse experiments

In the first season, Infected spike percentage recorded the values (0.00,0.00, 42.79, and 50.25) with cabbage treatment on cultivar Misr1, Misr2, Sids14, and Sakha95 but infected spike percentage recorded (0.00) with turnip and rocket While, the lowest value (0.00) was obtained with turnip, rocket and radish treatments on cultivars; Sids14 and Sakha95.

In addition, treatment with *Fusarium nygamai* increased the percentage of infected spikes in the wheat varieties Misr 2, Sids14, and Sakha 95 compared to the control treatment, which recorded a percentage of (100, 83.31, 100.00%), respectively, with the exception of the Giza 171 variety, which caused significant difference in the percentage of infected spikes for the biofumigation treatments (cabbage, turnip, rocket, radish) compared to the control treatment, which was greatly affected by the infection and did not show the emergence of spikes, while the Misr1 variety recorded an infection rate of 0.00 with the treatments of cabbage, turnip, rocket, radish the control treatment, which indicates the resistance of the variety to the *Fusarium nygamai*.

In the second season, Infected spike percentage recorded the value (0.00 and 0.00) with turnip and radish treatments on cultivars Misr1 and Misr2, but infected spike percentage recorded the highest value (47.39 and 16.35) with cabbage treatments on cultivars; Sakha95 and Giza171, respectively. while the lowest values (0.00 and 0.00) were obtained with rocket treatment on cultivars; Sakha95 and Giza171.

It was noted that the biofumigation treatments with cabbage and radish didn't affect the fungus, as no spike emergence was recorded in any of them with cultivars; Misr1 and Misr2, respectively. Also, the variety Sids14 did not record spike emergence in cabbage.

Biofumigation treatment with rocket succeeded in recording 0.00 with the wheat varieties Misr 1, Misr 2, Sids14, Sakha 95, and Giza 171, respectively, while the biofumigation treatment with turnip succeeded in recording the lowest percentage of infected spikes of 0.00 with the varieties Misr 1, Misr 2 and Giza 171, respectively, while the radish treatment succeeded with a percentage of 0.00 in the Giza 171 variety.

In the first season, Disease incidence recorded the highest value (100.00) with cabbage, turnip, radish, and control treatments on Misr1, Misr2, Sids14, Sakha95, and Giza171 cultivars. While the lowest value (77.76) was recorded with radish treatment in the second season.

Fig 3. A: Incorporating four Brassicaceae plants into the soil at maturity stage, **B***: Fusarium nygamai* inoculation on autoclaved barely grains**, C, D**: Biofumigation treatments.

Table 2 The effects of interaction between five wheat cultivars and biofumigation with four Brassicaceous crops. on Pathological characteristics of wheat plants cultivated in soil infested with *Fusarium nygamai* in two seasons 2021/2022 and 2022/2023:

Mean values of Pathological characters (cm)							
		Season 2021/ 2022			Season 2022/ 2023		
Cultivars	Treatments	FHB symptoms	FCR Crown root index		FHB symptoms	FCR Crown root index	
		After 90 days	After90days	After90 days	After 90 days	After90days	After90 days
		Infected spike percentage	Disease incidence	Disease severity	Infected spike percentage	Disease incidence	Disease severity
Misr1	Biocabbage	0.00	100.00	2.33	-	88.88	2.44
	Bioturnip	0.00	100.00	1.67	0.00	80.07	1.67
	Biorocket	0.00	100.00	1.50	0.00	69.97	1.11
	Bioradish	0.00	100.00	1.50	-	77.76	1.67
	Control	0.00	100.00	3.00	-	100.00	3.00
Misr3	Biocabbage	0.00	100.00	2.33	-	87.69	1.89
	Bioturnip	0.00	100.00	1.17	0.00	88.66	1.67
	Biorocket	0.00	100.00	1.00	0.00	88.88	0.89
	Bioradish	0.00	100.00	1.67	-	97.39	1.22
	Control	100.00	100.00	3.00	-	100.00	2.89
Sids14	Biocabbage	42.79	100.00	2.83	-	88.74	2.44
	Bioturnip	0.00	100.00	2.17	12.40	66.64	1.78
	Biorocket	0.00	100.00	2.50	0.00	66.64	1.56
	Bio-radish	0.00	100.00	1.33	62.51	77.76	2.22
	Control	83.31	100.00	3.25	-	100.00	3.39
Sakha95	Biocabbage	50.25	100.00	2.17	47.39	71.39	2.11
	Bioturnip	0.00	100.00	1.33	8.22	50.12	0.89
	Biorocket	0.00	62.47	0.75	0.00	37.44	0.83
	Bioradish	0.00	100.00	1.00	16.97	42.79	1.44
	Control	100.00	100.00	3.00	-	88.73	3.00
Giza171	Biocabbage	-	100.00	3.17	16.35	0.00	3.11
	Bioturnip	0.00	100.00	1.17	0.00	0.00	1.17
	Biorocket	0.00	100.00	1.33	0.00	0.00	1.33
	Bioradish	0.00	100.00	1.75	0.00	0.00	1.78
	Control	-	100.00	3.17	-	75.17	3.33
L.S.D (0.05)		**	**	**	**	**	**

(-) This means that un-infected spikes were zero.

In addition, *Fusarium nygamai* succeeded in causing disease incidence of wheat varieties Misr1, Misr2, Sids14, and Giza 171., except for the rocket treatment, which caused a disease incidence at rate (62.47) in the Sakha 95 variety (Table 2).

In the second season, Disease incidence recorded the highest values (88.88 and 71.39) with cabbage treatment on Misr1 and Sakha 95 cultivars. While the lowest value (66.64,37.44,0.00) was recorded with rocket treatment on Sids14, Sakha95, and Giza171 cultivars.

In addition, the treatment with *Fusarium nygamai* led to significant differences in the disease incidence rate of the biofumigation treatments of cabbage, turnip, rocket, and radish compared to the control treatment, which recorded 100% for Misr 1, Misr 2, and Sids14 varieties, respectively. However, control treatment in the Sakha 95 variety recorded a percentage of 88.73 while a significant difference was observed between the biofumigation treatments, which recorded 0.00 for treatments of cabbage, turnip, rocket, and radish, compared to the control treatment, which recorded 75.17 with Giza171 cultivar.

In the first season, Disease severity recorded the highest value (2.33, 2.33,2.83, 2.17, 3.17) with cabbage treatment on cultivars Misr1, Misr2, Sids14, Sakha95, and Giza171, respectively. While the lowest value (1.50, 1.00, and 0.75) with rocket treatment on cultivars; Misr1, Misr2 and Sakha95 but it recorded (1.50 and 1.33) with radish treatment on cultivars; Misr1 and Sids14.

In the second season, Disease severity recorded the highest value (2.44,1.89, 2.44,2.11,3.11) with cabbage treatment on cultivars Misr1, Misr2, Sids14, Sakha95, and Giza171, respectively while the lowest value (1.11, 0.89, 1.56 0.83, and 1.33) on cultivars; Misr1, Misr2, Sids14, Sakha95, and Giza171 respectively.

In both seasons, biofumigation treatments (cabbage, turnip, rocket and radish) succeeded in causing significant differences in disease severity compared to the control treatment in the varieties Misr 1, Misr 2, Sids14, Sakha 95, and Giza 171, respectively. In the 2021 season, biofumigation treatments (cabbage, turnip, rocket and radish) succeeded in causing a significant difference in the disease severity compared to the control treatment in the varieties Misr 1, Misr 2, Sids14, Sakha 95, and Giza 171, respectively.

Laboratory studies

Experiments to evaluate effects of biofumigation plant material on Fusarium nygamai seven days after incubation

The data in Table (3) indicated highly significant differences between treatments in both experimental seasons (2021/2022, 2022/2023). This study proved that all Brassica species tested reduced *Fusarium nygamai* growth after 7 days of incubation. Obtained data showed that macerated tissues of Rocket resulted in the highest mean values of mycelial growth reduction after 7 days of incubation 73.85 % and 73.90% in both studied seasons, respectively among all Brassica species. Then turnip resulted in mean values of mycelial growth reduction lower than Rocket after 3 days incubation 73.76 % and 73.52 % in both studied seasons respectively. However, Cabbage was the least effective treatment in suppressing the mycelial growth of *Fusarium nygamai* after 7 days of incubation 55.41 % and 55.54 % in both studied seasons respectively.

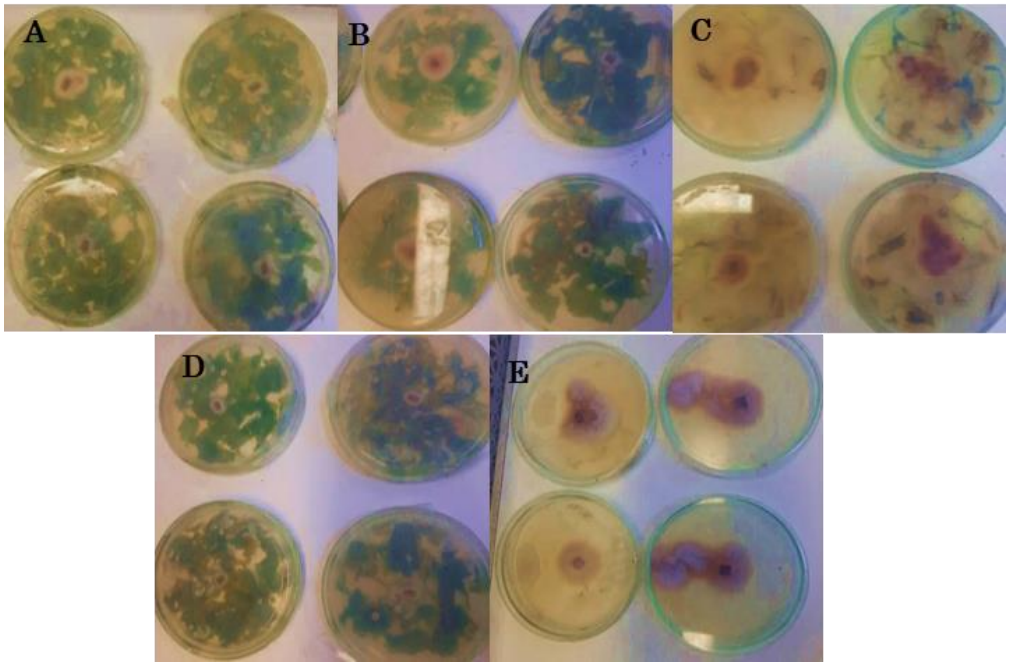

Fig 4. Application of biofumigation of brassicas against *Fusarium nygamai*. A: biofumigation by rocket, B: biofumigation by radish, C: biofumigation by cabbage, D: biofumigation by turnip, E: control.

Table 3 The effects of volatiles released from five species of Brassicaceae plants on *Fusarium nygamai* mycelial growth reduction %7 days after incubation in vitro.

Treatments	Linear growth (cm) after 7 days	Reduction (%)
	2021\2022	
Cabbage	3.13b	55.41 d
Radish	2.88 c	58.89 c
Rocket	1.83 e	73.85 a
Turnip	1.84 d	73.76 b
Control (untreated)	7.02 a	0.00 e
L.S.D 0.05	**	**
	2022\2023	
Cabbage	3.13 b	55.54 d
Radish	2.88 c	58.99 c
Rocket	1.83 e	73.90 a
Turnip	1.86 d	73.52 b
Control (untreated)	7.04 a	0.00 e
L.S.D 0.05	**	**

*Means in column followed by the same alphabetical letter are not significantly different at 5% level according to LSD.
*Each figure represents the mean value of four replicate.

Discussion

According to data presented in Table (1, 2), it is concluded that biofumigation with rocket treatments succeeded in inhabiting the linear growth and recorded the highest value of reduction of *Fusarium nygamai* in both seasons, and these results are consistent with Larkin and Griffin (2007) and Fan et al (2008) who noticed the ability of biofumigation plants in inhibiting growth of pathogenic fungi. Many researchers have given promising results with the use of biofumigation in the field. Koike and Subbarao (2000) concluded that using broccoli residues as a biofumigant plant for the management of *Verticillium* wilt of cauliflower was effective in reducing disease incidence and severity compared with control in the field.

In 003, Kirkegaard et al. found that Brassicaceous crops were the most efficient among other tested methods in decreasing crown rot infection of wheat. Motisi et al (2009) succeeded in using biofumigant plants as a biological method for controlling sugar beet root rot in the field. Read ford (2015) proved that rotation crops of brassica species successfully reduced the amount of crown rot inoculum compared to the fallow treatment during the growing season in the field in northern New South Wales. Drakopoulos et al. (2020) proved the capability of certain biofumigant plants to reduce mycotoxins produced by *Fusarium graminearum*, the causal agent of *Fusarium* head blight in wheat.in Egypt, Elsayed et al. (2022) recorded that biofumigation with *Brassica* plants can reduce disease incidence of head blight disease of wheat in Matrouh, Egypt. Also. Rubayet et al. (2018) found that biofumigation treatment inhibited the pathogen *Rhizoctonia solani* the causal agent of stem canker and black scurf diseases in potato.

Fusarium nygamai was first isolated by Burgess & Trimpoli (1986) from the roots of sorghum and subsequently from the head of grain sorghum, bean roots, and soil. Also, Onyike and Nelson (1992) were able to isolate *F. nygamai* from millet and sorghum from Nigeria, Zimbabwe, and Lesotho. While, Besharati Fard et al (2017) ensured that *Fusarium nygamai* was associated with wheat crown and root tissues in Eastern Iran. Minati et al (2020) succeeded in isolating *Fusarium nygamai* from diseased roots of wheat in Iraqi. Dehghanpour Farashah et al (2021) demonstrated that *Fusarium nygamai* was associated with crown rot of wheat in Iran. (Özer et al 2023) proved that *Fusarium nygamai* was associated with Crown rot of wheat in Northern Kyrgyzstan.

Minati et al. (2019) recorded for the first time 6 *Fusarium* species as causative pathogens of *Fusarium* head blight (FHB) and *Fusarium* crown rot (FCR) of the wheat crop in Iraq. *Fusarium nygamai* was one of these identified species. Minati et al (2020) found that almost all of the examined *Fusarium* species had significant effects on the discoloration of the basal stem of wheat plants as an indicator of *Fusarium* crown rot and *F. pseudograminearum* was considered the main pathogen of this disease as it recorded the highest rate of disease severity between the other isolated fungi. On the other hand, *Fusarium culmorum, Fusarium cerealis,* and *Fusarium graminearum* reigned supreme in causing

Fusarium head blight three weeks after inoculation, while *F. chlamydosporum* and *F. nygamai* displayed the lowest effectiveness.

In 2006, Balali and Iranpoor demonstrated that the differences in disease causal agent from one region to another were due to the large genetic variation in the Fusarium genus because it is widespread in the environment and can adapt to unfavorable environmental conditions. Therefore, new strains of Fusarium may emerge due to environmental pressure and the development of mutations so significant differences appeared between isolates of the same species of *Fusarium*. Also, various investigators (Strausbaugh et al. 2005; Moya 2010; Stępień & Chełkowski 2010) stated that the reason for this difference between *Fusarium* isolates in their ability to infect wheat seedlings can be due to genetic differences in the genes controlling the pathogenicity and other factors such as temperature, humidity, etc.

The findings of Leyva-Madrigal et al. (2015) demonstrated the ability of the *Fusarium nygamai* between the other three species of *Fusarium* to infect maize seedlings and induce different levels of disease severity and stated that *Fusarium nygamai* could infect maize roots in Mexico and was able to colonize every root tissue, including the epidermis and vascular vessels. They also found that the highest disease severity of any examined Fusarium isolates from maize roots was recorded for *F. nygamai* (90.6) %. Similarly, this species has been isolated from maize ears in India (Chehri 2011).

In addition, Shrestha et al. (2021) noticed that *Fusarium nygamai*, was found in 44 isolates collected across eight fields in Bishkek and one in Sokuluk, in North Dakota, USA. This represents 30% of the surveyed sites and an isolation frequency of 7.36%. Zhang et al. (2023) reported the ability of *Fusarium nygamai* in causing Crown Rot of Wheat in China.

Results presented in table 2 are consistent with the findings of Zukalová et al. (2003) and Swetha, et al (2020). They emphasized that glucosinolates act as natural biofumigants and biofumigation is a soil disinfection method without chemicals. This process leads to alterations in the physical, chemical, and biological properties of the soil ultimately contributing to the suppression of pathogens Kirkegaard and Matthiessen, (2004), Cohen et al., 2005, Matthiessen and Kirkegaard (2006), Stark et al., (2008), Larkin et al., (2007).

Conclusion

This study identified an isolate of Fusarium nygamai and deposited it in GenBank under accession number OQ152508. Pathogenicity assessments on six wheat cultivars (Misr1, Misr3, Giza 171, Sids 14, Egaseed 22, and Sakha 95) revealed that Sids 14 and Sakha 95 exhibited the highest resistance to crown rot disease in North Sinai conditions. Research conducted in greenhouses and in vitro demonstrated that biofumigation utilizing rocket treatment is the most effective method for managing FCR and FHD diseases in wheat in North Sinai, while also ensuring environmental safety.

Conflict of interest

The authors declare that they have no conflict of interest.

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