International Journal of Innovative Research and Scientific Studies, 8(1) 2025, pages: 834-840

IJIRSS International Journal of Innovative Research & Scientific Studies

ISSN: 2617-6548

URL: www.ijirss.com

Fungicidal properties of green microalgae chlorella vulgaris

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Abstract

Mold fungi are a group of microscopic fungi that have a significant impact on agriculture. They not only worsen the quality of products, but also reduce yields due to damage to plants and seeds. Studying the harm caused by mold is an important task of agronomic science, as it contributes to the development of effective control and prevention methods. The presented article examines the results of the influence of Chlorella vulgaris strains of green microalgae on mold fungi common in vegetable crops. Studies have shown that the use of biofertilizers based on Chlorella vulgaris is environmentally friendly due to their minimal accumulation in the soil. In addition, these fertilizers are economically beneficial, since man-made waste products such as phosphorous slags, sewage sludge, and carbon-containing waste can be used to grow biomass of microorganisms and algae. In the course of research, it was found that fertilizers based on Chlorella vulgaris inhibit the growth of mold fungi Mucor sp. and Aspergillus sp. in the soil and on the surface of seeds of agricultural crops. Compared with traditional fertilizers, their effectiveness reaches 28.6±2.2%. These results confirm the promising use of Chlorella vulgaris to protect crops from mold fungi, improve product quality and increase yields.

Keywords: Chlorella vulgaris, Fertilizers, Fungicides, High yield, Microalgae soil microbiology, Vegetable crops.

DOI: 10.53894/ijirss.v8i1.4428
Funding: This research is supported by KS MSHE RK (Grant number: AP22684726).
History: Received: 27 November 2024/**Revised:** 3 January 2025/**Accepted:** 17 January 2025/**Published:** 31 January 2025

Competing Interests: The authors declare that they have no competing interests.
Authors' Contributions: All authors contributed equally to the conception and design of the study. All authors have read and agreed to the published version of the manuscript.
Transparency: The authors confirm that the manuscript is an honest, accurate, and transparent account of the study; that no vital features of the study have been omitted; and that any discrepancies from the study as planned have been explained. This study followed all ethical practices during writing.
Publisher: Innovative Research Publishing

1. Introduction

Mold fungi are one of the significant problems during storage and sowing of vegetable crops, as they affect both the quality of the product and its safety. Their harm is manifested in the following aspects:

Crop losses. Mold contributes to the spoilage of vegetables during storage, reducing their commercial quality and nutritional value. The main pathogens are representatives of the genera *Aspergillus*, *Penicillium* and *Fusarium*. These microorganisms develop at high humidity and violation of the temperature regime [1].

Release of mycotoxins. Some mold fungi release toxic substances known as mycotoxins (for example, aflatoxins and patulins), which pose a danger to human and animal health. Their presence reduces the suitability of vegetables for consumption and can cause acute or chronic poisoning.

Decreased seed germination. Infection of seeds with mold during storage leads to a decrease in their germination. Fungi of the genus *Fusarium* and *Alternaria* cause necrosis of seed tissue, which impairs their ability to germinate and grow further [2].

Development of phytopathogens. Some mold fungi are phytopathogens that cause diseases of vegetable crops, such as fusarium, alternaria and root rot. These diseases can cause plant death or a significant reduction in yield [3].

Acceleration of putrefactive processes. Mold promotes the decomposition of plant tissue, which accelerates the development of bacterial rot. This is especially true for vegetables with thin skins, such as tomatoes, cucumbers or peppers.

According to research to minimize damage from mold, it is necessary to: maintain optimal storage conditions (humidity 70-85%, temperature 0-4 °C, depending on the crop); treat seeds with fungicides before sowing; ensure regular ventilation of storage facilities; remove contaminated vegetables and seeds to prevent the spread of fungi [4] mold control at all stages of storage and sowing therefore plays a key role in ensuring food safety and maintaining the quality of vegetable crops [5].

A wide range of fungicides are used to prevent mold damage to vegetable crops and improve seed preservation. Their choice depends on the type of crop, the type of pathogen, and the stage of treatment. Here are examples of commonly used fungicides:

1. For seed treatment before sowing. This treatment prevents fungal infection in the soil and stimulates healthy germination [6].

Thiram (TMTD). A broad-spectrum contact fungicide that protects seeds from mold and soil fungi. It is used for cereals, vegetables, and industrial crops.

Carboxin + Thiram (*Vitaros, Fundazim*). A combination product that is effective against root rot and other diseases caused by mold fungi.

Fludioxonil (Maxim). A modern contact fungicide with prolonged action. Prevents the development of mold, fusarium, and other diseases.

2. To protect plants during growth. Treatments of vegetative plants help prevent secondary infection [7].

Azoxystrobin (Quadris). A systemic fungicide effective against a wide range of fungi, including mold.

Propiconazole (Tilt). A systemic fungicide that penetrates into plant tissues, effective against fusarium, powdery mildew and spotting.

Chlorothalonil (Bravo). A contact fungicide that prevents the spread of mold on the surface of plants.

3. To protect during storage. During storage of vegetables, fungicides help prevent the development of mold and bacterial rot [8].

Imidazoles (Imazalil) (Fundanol). Efficient for treating fruits before storage, prevents infection of citrus fruits, tomatoes and potatoes.

Pyrimethanil (Skor). Used to prevent gray mold, which is common in storage facilities.

Fenhexamid (Teldor). Used to treat vegetables and fruits before storing.

Biological fertilizers are an important element in modern agriculture, as they have a number of advantages over chemical analogues. One of the main advantages of biological fertilizers is their ability to improve the soil structure and enrich it with microorganisms, which helps to increase soil fertility [9]. This, in turn, helps to increase crop yields and improve the quality of products.

Biotechnology in agriculture, especially in the field of crop farming, offers many methods for increasing soil fertility. The problem of improving the efficiency of agricultural production remains relevant [10]. One area that deserves attention is the introduction of bacteria into the soil that can effectively enrich it with bioavailable nitrogen and phosphorus [11]. For example, some studies add nitrogen-fixing and phosphate-mobilizing microorganisms to the soil, as well as ammonium nitrate and phosphate rock, both in regular and nanosized form. Ammonium nitrate is a popular source of nitrogen, while phosphate rock is an effective and safe mineral supplement with a long-term effect [12].

In addition, biological fertilizers are a more environmentally friendly and safer option for the environment and human health, as they do not contain harmful chemical components [13]. This is especially important in the context of sustainable agricultural development and the preservation of biodiversity [14].

An example of comparison is the effect of mineral fertilizers and biological fertilizers on crops. Research shows that the use of biological fertilizers contributes to more uniform plant growth, increased resistance to stress conditions and improved nutritional composition of fruits and vegetables [15]. In addition, biological fertilizers help maintain the biological balance in the soil, which has a positive effect on long-term productivity and soil resistance to erosion [16]. Biological fertilizers based on algae are an effective and environmentally friendly way to improve soil fertility and increase crop yields. Algae contain many nutrients and biostimulants that promote active plant growth and development [17].

Below are the names of some algae-based biofertilizers and their applications:

1. "Algamik" is a biopreparation based on seaweed extract, which contains microelements, amino acids, vitamins and growth hormones. It is used to stimulate plant growth, improve immunity and increase crop yields.

2. "Fitospirin" is a biohumate fertilizer based on algae extract, which helps improve soil structure, activate microorganisms and increase plant resistance to stress conditions.

3. "Silit" is an algae-based biostimulant that contains phytohormones and amino acids. It is used to increase seed germination, stimulate root system growth and improve nutrient metabolism in plants.

Algae-based biofertilizers are an effective means of improving soil fertility, stimulating plant growth and development, and increasing crop yields [18, 19]. Their use promotes the growth of green mass, improves crop quality and preserves the environment due to the absence of chemical additives [20].

Chlorella is a microscopic freshwater algae belonging to the class of green algae, which is widely used not only as a food additive, but also as a biological fertilizer in agriculture. Its high content of protein, vitamins, minerals and other nutrients makes it a valuable source of nutrients for plants [21].

Using chlorella as a fertilizer has several main advantages:

1. Soil enrichment: chlorella contains a huge amount of nitrogen, phosphorus, potassium and other nutrients that help improve soil fertility and provide plants with essential nutrients [22].

2. Growth stimulation: biologically active compounds contained in chlorella help accelerate plant growth, increase yields and improve product quality [23].

3. Improvement of soil flora: chlorella helps activate microorganisms in the soil, which contributes to more efficient synthesis of nutrients and increases plant resistance to stressful conditions [24]. Thus, the use of chlorella as a fertilizer can be an effective way to increase the yield and quality of agricultural products, as well as improve the environmental sustainability of the agricultural system.

Our previous studies have shown that green microalgae chlorella can utilize waste from phosphorus production. As a result of these studies, a biofertilizer was obtained that increases the yield of agricultural crops [25].

Currently, many new biological fertilizers with various properties appear, which are better and cheaper than chemical analogues. Unfortunately, there are no studies on the fungicidal effect on plant seeds and soil. Mold of agricultural seeds is one of the main problems. Due to the damage of the initial stage of seed storage, the germination of plants decreases, and micromycete spores pollute the soils of crop lands.

In this regard, the purpose of our study was to study the fungicidal properties of algal fertilizer based on green chlorella microalgae.

2. Materials and Methods

The subjects of the study included strains of algae, strains of micromycetes, and agricultural crops (carrots, soybeans, beans). The Chlorella vulgaris strain was isolated from the Koshkar Ata River in southern Kazakhstan. Micromycete strains were extracted from the soil of agricultural lands.

Microscopic preparations were examined using a Biomed-5 microscope equipped with 10×, 40×, and 100× objectives, as well as immersion oil in accordance with GOST 28489-90. The analysis of water samples was performed using the "crushed drop" technique with defatted cover slips and glass slides measuring 75×25 mm and 18×18 mm. Pipettes with diameters of 1 mm and 2 mm were utilized, and a dilute potassium iodide (KI) solution was used to immobilize motile aquatic organisms for fixation.

Taxonomic identification was carried out based on specialized identification guides. Video documentation of the observed specimens was performed using a Samsung Digimax S600 camera. Microorganisms were cultivated on appropriate liquid and solid selective media using methods such as Koch's technique, Novogrudsky's method, "exhaustive seeding," and limiting tenfold dilution for microbiological cultures. Pure cultures were obtained on slanted nutrient agar. Nutrient media and glassware were sterilized using a bacteriological autoclave (SPGA-100-I-HH). Cultivation of microorganisms was conducted in a programmable thermostat (TC 1/80) within a prepared microbiological cabinet.

The selective media were prepared following GOST R 51758-2001 guidelines:

Tamiya medium: KNO_3 - 5.0 g; $MgSO_4 \times 7H_2O$ - 2.5 g; KH_2PO_4 - 1.25 g; $FeSO_4 \times 7H_2O$ - 0.003 g; microelement solution - 1 ml; EDTA - 0.037 g.

Prat medium (g/l): KNO_3 - 0.10 g; $MgSO_4 \times 7H_2O$ - 0.01 g; K_2HPO_4 - 0.01 g; $FeCl_3 \times 6H_2O$ - 0.001 g; agar-agar - 12 g.

ITA medium (g/l): phosphorus-containing slags - 10 g; KNO_3 - 0.10 g; $MgSO_4 \times 7H_2O$ - 0.01 g. Phosphorus slags used in the medium contained P_2O_5 - 0.23 g/l, nitrogen - 0.06 g/l, and potassium - 0.04 g/l, and were ground to a particle size of 0.1-0.2 mm using a mechanical grinder.

In the control group, test plants were irrigated with tap water. In experimental groups, solutions containing different concentrations of phosphorus-containing waste and algae were applied.

Bacterial taxonomic classification was performed using Bergey's Manual of Determinative Bacteriology, while micromycetes were identified using the Identifier of Pathogenic and Opportunistic Fungi. The morphology of micromycetes was evaluated based on colony characteristics on Petri dishes, including shape, cross-sectional appearance, edges, texture, color, and pigment diffusion into the agar medium.During the preparation of the nutrient medium, "Scout-Pro" brand scales (GOST 24104-2001) were used, and sterilization was done using the SPGA-100-1-NN autoclave No. 141. To determine the bacterial titer, a 1 g sample obtained after quartering was mixed in 100 ml of water on a shaker for 30 minutes. The resulting suspension was diluted in the nutrient medium using a tenfold dilution method. 1 ml of the sample suspension was cultivated on the nutrient medium. Microorganism cultivation occurred at 25°C for 7 days. Laboratory glassware and nutrient media were sterilized in an autoclave (SPGA-100-1-NN No. 141). Colonies were counted after 72 hours.

Algae were cultivated in a sterile box on solid Myers and Prat nutrient media (Figure 1).

Figure 1.
Pure strain of chlorella vulgaris.

The incubation of cultures grown on liquid nutrient media was conducted on light racks (No. GS-1/80 SPUTU 9452-002-00141798-97) at a temperature of +23 to +25°C. The accumulating culture of microalgae was grown on light racks in plastic containers with a volume of 5 liters at a temperature of +23 to +25°C with continuous aeration. The concentrations of Pb^{2+}, Cd^{2+}, Cu^{2+}, and Zn^{2+} ions in the aquatic environment were determined using the STA-1 complex, employing the inversion voltammetry method and atomic absorption spectroscopy method on an AAS 1 spectrophotometer. Chloride, sulfate, nitrate, and nitrite ions were measured using the photocolorimetric method on a KFK-3-01-ZOMZ photometer and the ionometric method on an I-500 ion meter.

Percentage suspensions were prepared according to GOST 4517-87. Suspensions of 5.0 vol.%, 10.0 vol.%, 15.0 vol.%, and 20.0 vol.% of slag and sludge were produced, in which microalgae strains were cultivated for 7 days. The studies utilized Prat medium and ITA medium with the addition of phosphorus-containing waste (10.0 ± 0.1 g of phosphorus-containing waste was added instead of 10.0 ± 0.1 g of dibasic potassium phosphate). Cultivation occurred for 7 days at a temperature of +23.0 ± 1.00°C with a 12-hour light period. For sowing test plants, disposable plastic cups containing 50.0 ± 0.1 g of sterile expanded vermiculite were used, where calibrated seeds of test plants inoculated with Chlorella were planted. To maintain moisture exchange, the cups were tightly covered with film.

Morphometric data, including plant length, stem and root lengths; weight indicators were measured with a ruler and analytical balance. The number of leaves on seedlings was counted visually.

The qualitative composition of lipid extracts from microalgae was determined using thin-layer chromatography (TLC) with hexane-ethyl ether-acetic acid (80:20:1) as the eluent. The analysis of lipid composition was conducted using GC/MS on an Agilent 7000B device in the Department of Chemistry at Adam Mickiewicz University in Poznań.

Statistical processing of the obtained results was carried out through the calculation of arithmetic means and standard deviations. All studies were conducted in a five-fold sequence. The data were processed using a personal computer with the Excel application package.

3. Results and Discussion

It is well known that seed storage is a critical stage in agronomy and horticulture, since the quality of storage affects the germination and health of plants. One of the most serious threats to seeds is the development of mold, which can lead to a decrease in their viability and deterioration in the quality of the crop. Mold on seeds occurs as a result of microbiological processes caused by specific storage conditions. The main factors contributing to the development of fungal infections include: high humidity promotes the reproduction of fungal spores, improper temperature conditions, increased oxygen availability, mold can develop on seeds already infected with fungal spores before storage.

Violation of these factors on seeds can lead to the following negative consequences: decreased germination, loss of nutrients, accumulation of toxic metabolites.

The problem of mold in the storage and sowing of seeds is a significant risk to agriculture. The use of modern methods of storage, seed treatment and storage conditions control can significantly reduce the likelihood of mold development and preserve the quality of seeds, which in turn will ensure a high yield and plant resistance. The study established the ability of fertilizer based on Chlorella vulgaris to suppress the development of mold fungi *Mucor* sp., *Aspergillus* sp. on the seeds of test plants. During additional experiments, results were obtained on the study of the fungistatic abilities of algae.

In the course of our previous studies, it was noted that in samples of test plants with the addition of microalgae culture to the soil, the mold formation of seeds decreases.

The effect of *Chlorella* sp. algae on mold fungi is of interest in the context of biological interactions and possible applications in agriculture and the food industry. The interaction between chlorella and mold fungi is complex and depends on many factors, including environmental conditions and specific types of organisms. Chlorella shows potential in suppressing the growth of mold fungi due to its antimicrobial properties, which can be useful in the storage of agricultural products. In one study, it was shown that treating apples with chlorella suspension delayed mold development for up to 41 days, while mold appeared in the control group as early as day 17[26].

Another study described a symbiotic association between chlorella, bacteria, and fungi, where fungi and bacteria promote the growth of chlorella, providing it with essential nutrients and protecting it from adverse conditions. On the other hand, chlorella can act as a biocontrol agent against phytopathogenic fungi. A study published in the journal Plants has shown that *Chlorella vulgaris* effectively inhibits the growth of *Fusarium oxysporum* fungus, reducing the incidence of spinach [27].

During the additional experiments, the results of studying the fungistatistical abilities of algae were obtained (Figure 2).

Figure 2.
Comparative analysis of fungicidal properties of algal fertilizer.

A- bean seeds without treatment, a- bean seeds after treatment with algal fertilizer.
B- soybean seeds without treatment, b- soybean seeds after treatment with algal fertilizer.

In laboratory experiments, kidney bean (Phaseolus vulgaris) and soybean (Glycine max) seeds were selected as test plants. The studies were conducted to assess the level of seed mold when using various fertilizers, including traditional ammophos and Chlorella vulgaris ASLI-1. The experiment included a week of incubation of non-sterile beans under conditions conducive to the development of mold fungi. In the control variant, where ammophos was used, the beans were subject to intense mold. According to the analysis results, on average 95.2% of the seeds were covered with mold (with an error of ±2.3%). This figure can be considered very high, indicating weak protection of seeds from mold fungi when using this fertilizer. In the variant with *Chlorella vulgaris*, the results were significantly better. The average level of mold was only 12.3% (with an error of ±0.4%). This indicates a significant suppression of mold growth due to the use of this fertilizer. Thus, the difference in mold levels between the two options was 82.9%. These data confirm the effectiveness *of Chlorella vulgaris* in protecting seeds from mold fungi, which makes it promising for use in agricultural practice.

Additional experiments to study the fungicidal properties of algal fertilizer were conducted using ripe fruits of *Daucus carota*. Ripe fruits were treated in water (A), ammophos (B), algal fertilizer (C) and incubated for a week in a closed container (Figure 3).

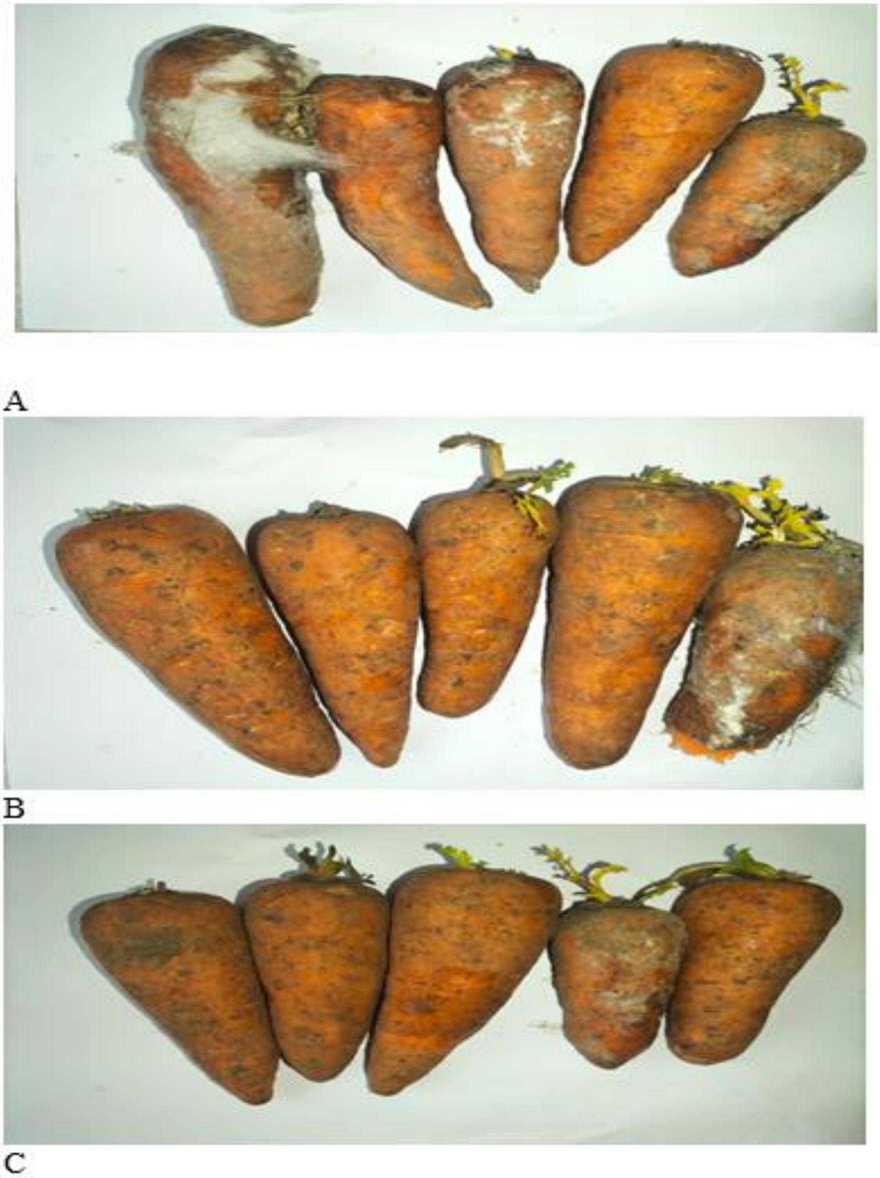

Figure 3.
Study of fungicidal properties of algal fertilizer on carrot fruits.

Analysis of fungal contamination and structural integrity across treatment groups revealed the following results: The control variant (H_2O treatment) exhibited fungal growth covering 68.7 ± 3.7% of the fruit surface area, while the ammophos-treated specimens showed reduced fungal coverage of 29.4 ± 2.3%, though accompanied by notable tissue degradation and onset of vegetative decomposition. In contrast, specimens treated with algal fertilizer demonstrated the lowest fungal contamination at 18.1 ± 0.9% of surface area, with the additional benefit of maintaining structural integrity with no observable morphological alterations in the root crops.

4. Conclusion

The interaction between *Chlorella* and mold fungi is complex and depends on many factors, including environmental conditions and specific types of organisms. *Chlorella* shows potential in suppressing the growth of mold fungi due to its antimicrobial properties, which can be useful in the storage of agricultural products.Thus, according to the results of the study of the effect of fertilizer based on green microalgae on the seeds of agricultural crops, it was found that fungicidal properties suppress mold growth by an average of 28.6±2.2%.The obtained research results show that algal fertilizers increase crop yields not only due to nutrients, but also due to protection of seeds from mold. Thus, fertilizers based on green microalgae can be used to restore soils with a high content of mold fungi. Since there is not enough research in this area, our research can be the basis for further development of this area.

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