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Effective Control of Fusarium Wilt and Root Rot in Cucumber Plants Using Mustard Seed Extract as a Soil Bio-Fumigant

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Abstract

Cucumber (*Cucumis sativus* L.) belongs to the Cucurbitaceae family. It is characterized by its rich flavor and essential nutrients, including vitamins and minerals, especially potassium and magnesium. Fusarium wilt is one of the most serious plant pathogens, posing a significant challenge to cucumber production. This disease can severely damage cucumber plants and lead to significant economic losses. Various strategies have been employed to control the detrimental effects of cucumber root rot. The most common is the use of fungicides. However, the use of fungicides causes environmental pollution, and as a result, several strategies have been explored, such as the use of resistant cucumber varieties, plant extracts, or chemical control techniques. In this study, we focus on the effect of mustard seed extract as a bio-fumigant on the growth of the fungi responsible for wilting cucumber plants.

The green manures derived from Brassicaceae crops have demonstrated efficacy in controlling Fusarium wilt diseases in crops. The powdered Brassica nigra seeds were used to prepare an aqueous extract to determine their chemical composition, mineral content and antioxidant properties. Analysis revealed that mustard seeds contained 28.0 g of protein, 36.0 g of carbohydrates, and 8.7 g of tannins per 100 g. The findings indicated that mustard seeds are rich in various essential mineral nutrients. The mustard seeds possess significant

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antioxidant activity, achieving an inhibition percentage of 75.2%. These bioactive compounds are beneficial in alleviating oxidative stress and lowering the likelihood of disease occurrence. The study conducted a biochemical analysis of mustard seeds, revealing the presence of anti-nutritional compounds, with a measured Sinigrin content of 51.6 mg/g and glucosinolate content of 12.4 mg/g in mustard.

The sterilized seeds of black mustard, Brassica nigra, were germinated on MS medium supplemented with GA_3 at varying concentrations (0.1, 0.5, 1.0, 2.0 and 3.0 mg/l). An increase in GA_3 concentration corresponded to a higher germination rate. For callus induction, cotyledons and hypocotyls from uniformly sized (1 cm) *in vitro* grown seedlings were employed as explants and cultured on MS medium supplemented with various concentrations of 2,4-D with BA at (0.5 mg/l) for two months. Callus formation from cotyledon explants ranged from approximately 25.0 to 84.6%. Additionally, callus formation from hypocotyl explants was observed to be between 10.0 and 40.5%. To establish embryogenic cell suspension cultures, pieces of friable callus, aged eight weeks, the cultures from both the hypocotyl and cotyledon were moved to a liquid medium with 1.0 mg/l of 2,4-D and 0.5 mg/l of BA, where they were maintained for two months on a horizontal shaker. This extract was subsequently tested against the mycelial growth of *Fusarium sp*., which was cultivated on PDA medium.

The incidence of Fusarium root rot in cucumber and the severity percentages of root rot were evaluated after 60 days post-planting under greenhouse conditions during the growing season at the Mohamed Naguib Air Base, El Alamein Road, Egypt. Approximately 20 infected roots were collected from cucumber plants showing symptoms of wilt and root rot. The infection symptoms included leaf yellowing, root decay and brown discoloration of the roots. These specimens were transported to the Plant Pathology Department at the National Research Centre in Dokki, Egypt, for isolation. The fungi that were isolated were identified as *Fusarium solani* and *Fusarium oxysporum*, based on the morphological characteristics of the fungal hyphae and conidia.

The *in vitro* mustard extract from suspension cultures was utilized at various doses of 2, 4 and 8 ml as an antifungal growth on PDA medium. Inoculant preparation and soil inoculation involved the preparation of the mustard inoculant utilizing a steam-sterilized sand-mustard medium at a ratio of 1:3. The inoculum was added to the soil at rates of 0.5 g, 1.0 g and 2.0 g per plant, and ensuring thorough mixing of the inoculum into the soil. In another experiment, spray the infected plants with Teko 8% WDG fungicide at a rate of 250 g/100 liters of water once. Treat the soil by making a solution of 10 g of Tico compound per 5 liters of water. Evaluation effect of sand-mustard medium and Teko 80% WDG fungicide on pathogenic fungal growth, fusarium wilt of cucumber plants under greenhouse conditions, productivity and fruit quality was measured after 9 and 15 days of treatments.

Keywords: *Fusarium oxysporum, Fusarium solani*, cucumber plants, bio-fumigant, cell suspension culture, mustard seed extract and chemical composition.

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Introduction

Cucumber (*Cucumis sativus* L.) is a member of the Cucurbitaceae family. It can be grown in both open fields and greenhouse environments. Fresh cucumber fruits play a crucial role in human nutrition and health because of their taste and essential nutrients, including vitamins and minerals, especially potassium and magnesium. Furthermore, as noted by **Arul *et al*. (1994)**, cucumbers are a significant source of fiber, complex carbohydrates, antioxidants and anti-carcinogenic compounds.

Regrettably, cucumber, one of the most important vegetable crops globally, faces numerous diseases in the field, leading to substantial yield losses **(Mohammed and Hasan 2018)**. In Egypt, the production of cucumbers is severely impacted by root rot disease, which is caused by various pathogenic fungi present in the soil.

Plant pathogens primarily include fungi, bacteria and viruses. While fungi represent the largest group of pathogens, the majority of plant diseases are attributed to viruses or bacteria. This is due to the ability of viruses and bacteria to infiltrate and disseminate through plant veins, a process referred to as systemic infection. Conversely, fungi are generally restricted to the outer layers of the plant, where infections initiate through airborne spores and subsequently spread by attacking cells adjacent to these outer layers **(Pranjal *et al*., 2024)**. Soil-borne plant diseases occur when pathogens in the soil infect plants through their roots. *F. solani* and *F. oxysporum* are examples of such soil-borne pathogens. These pathogens can survive in the soil for long durations as chlamydospores, infiltrate the roots, spread into the plant tissues, colonize and metastasize within the xylem vessels, leading to systemic yellowing, wilting, and ultimately, plant death **(Arie, 2019).**

Crop diseases represent a major threat to global food security **(Chakraborty and Newton, 2011)**. Fusarium is recognized as one of the most important groups of plant pathogens, known for causing severe diseases in various economically essential plants, such as cucumbers, watermelons, and tomatoes, among others **(Ajmal *et al*., 2023)**.

The Fusarium wilt disease affecting cucumbers has appeared as a significant challenge in cucumber cultivation. This affliction can severely damage cucumber plants, leading to substantial economic repercussions for farmers. The disease is widespread across numerous countries, with yield reductions estimated between 40% and 70% **(Sharma and Shukla, 2021)**. **Fareed *et al*. (2017)** note that cucumber wilt is prevalent in many of the global regions where cucumbers are cultivated.

The primary agents responsible for common cucumber root rot diseases include *Fusarium solani, Fusarium oxysporum, Rhizoctonia solani, Macrophomina phaseolina, Sclerotium rolfsii* and *Sclerotinia* spp. **(Elwakil *et al*., 2015)**. Various management approaches have been implemented to mitigate the adverse impacts of root rot disease in cucumbers. One of the most widely recognized strategies involves the application of fungicides. Nevertheless, the use of fungicides carries risks of environmental contamination, leaves toxic residues, and may contribute to the development of resistant pathogens **(Vinale *et al*., 2008)**. Consequently,

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numerous alternative strategies have been explored, including the utilization of resistant cucumber varieties **(Borrelli *et al*., 2018)**, plant extracts **(Han *et al*., 2018)**, and chemical control methods **(Karim *et al*., 2018)**.

Fusarium oxysporum and *Fusarium solani* cause root rot and damping-off in cucumber plants, leading to reduced yields. Pathogenicity tests have shown that the main symptom of Fusarium wilt disease is the yellowing of older leaves. As the disease advances, the affected plant will wilt and eventually die **(Din *et al.,* 2020)**. The wilting is also linked to chlorosis, necrosis in the interveinal regions of the leaves and an inability to produce fruit. Subsequent infections can lead to the production of malformed fruits **(Al-Tuwaijri, 2015)**. Fusarium wilt is a major factor that limits yield in cucumber cultivation **(Zhou *et al*., 2017)**.

The green manures derived from Brassicaceae crops have demonstrated efficacy in managing Fusarium wilt diseases in crops **(Mowlick *et al*., 2013)**. Mustard holds a significant role among spices due to its diverse array of phytochemicals. Additionally, it is recognized as one of the oldest oilseed crops, with global cultivation records tracing back to 3000 BC **(Golubkina *et al*., 2018)**. Prominent varieties of mustard consist of *Brassica nigra*, black mustard, *Brassica juncea*, brown mustard, white mustard and *Brassica rugose* **(Rahman *et al.*, 2018)**. Mustard is generally cultivated in sandy loam soils with low rainfall and is mainly grown in temperate climates, though it can also thrive in tropical and subtropical regions. It is regarded as one of the first domesticated crops, commonly found in Asia, North Africa, and Europe **(Divakaran and Babu, 2016).** Black mustard is a wild variety that naturally grows in the plains and hilly areas of the Mediterranean. Prior research has indicated that it may exert an allelopathic influence on certain crop plants **(Turk and Tawaha, 2002)**.

Brassica nigra was assessed for its capacity to inhibit plants pathogens through the volatiles released from its leaf tissue. The mustard generates compounds that impede the radial growth of fungi. The treatment of mustard that released over 1.2 mg of AITC per gram of plant tissue exhibited fungicidal properties **(Aguilar-González *et al*., 2015)**. The mustard seeds demonstrated inhibitory effects against all tested fungal strains **(Reyes-Jurado *et al*., 2019)**. The Brassicaceae family, classified under the order Brassicales (previously referred to as Capparales), includes more than 330 genera and over 3,700 species globally. A distinctive characteristic of this family is the presence of four sepals at the center of the flowers, accompanied by four alternate petals arranged in a cross formation, which reflects the historical name of the family, Cruciferae. Additionally, the presence of organosulfur compounds, particularly glucosinolates, is a notable feature of this plant family **(Pranjal *et al*., 2024)**. Plants serve as a significant source of various secondary metabolites, which exhibit a broad spectrum of pharmacological effects **(Al-Snafi, 2015)**.

Mustard seeds are rich in different bioactive compounds, like omega-3 fatty acids and glucosinolates **(Melrose, 2019)**. Omega-3 fatty acids, which are unsaturated fatty acids, are widely recognized for their health advantages **(Gammone *et al*., 2019)**. Numerous glucosinolate compounds have been isolated from mustard seeds. The primary glucosinolates

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obtained from mustard seeds include sinigrin and sinalbin **(Grygier, 2022)**. Glucosinolates are the most extensively researched defense-related secondary metabolites within the cruciferous family. These compounds are secondary products derived from amino acids, characterized by the presence of a sulfate group and thioglucose, and are predominantly found in the order Capparales **(Halkier and Gershenzon, 2006)**. They represent a largely homogeneous group of naturally occurring, anionic, hydrophilic, non-volatile and predominantly water-soluble compounds. During hydrolysis, glucosinolates generally release D-glucose, sulfate, and an unstable aglucone, which subsequently rearranges to yield isothiocyanate as the primary product, along with thiocyanate or nitrile (organocyanide) as secondary products.

Glucosinolates serve primarily as defensive agents against insect pests, with their concentration increasing in response to insect damage, leading to diverse effects on insects. They can function as both inducers and deterrents **(Hopkins *et al*., 2009)**. The insecticidal properties of glucosinolates arise from alterations in insect metabolism, particularly through the inhibition of the Krebs cycle of glycolysis, which results in decreased total oxygen uptake and carbon dioxide output.

The allelopathic impacts of various Brassica species on other plant species have been documented. Numerous putative allelopathic compounds have been extracted from Brassicaceae, and their allelopathic efficacy has been validated through bioassays. It is crucial to ascertain the optimal concentrations at which allelopathic responses manifest in weed management strategies. Additionally, different plant parts exhibit varying degrees of allelopathic potential **(Chun and Kim, 2002)**. The mustard plant is exceptionally valuable among medicinal plants due to its ability to target specific microbes in various ways through cellular expression by releasing different types of molecular proteins **(Jankowski *et al*., 2014)**. Glucosinolates released into the rhizosphere are effective in eliminating plants, nematodes, insects, and fungi **(Yu and Morishita, 2014)**.

This research aimed to assess the effectiveness of black mustard extract as a bio-fumigant in managing Fusarium wilt disease in cucumber plants, both in *in vitro* and greenhouse condition.

2. Materials and Methods

The experiments was carried out in greenhouse conditions at the Mohamed Naguib Air Base, located on El Alamein Road, Egypt, during the growing seasons of 2024 and 2025. The study achieved in Biotechnology Lab of Plant Cell and Tissue Culture in Breeding Department, Horticulture Research Institute, Agricultural Research Center, Giza, Egypt. All analysis achieved in Chemistry of Natural and Microbial Products Department, National Research Centre, Dokki, Egypt. The Fungi isolated achieved in Plant Pathology Department, National Research Centre, Dokki, Egypt.

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Experiment 1

2.1- Extract preparation

Mustard seeds **(Fig. 1)** were washed completely with water. Subsequently, the seeds were air-dried for a period to eliminate excess moisture. An aqueous extract was created by gradually mixing 100 g of powdered *Brassica nigra* seeds with 250 ml of distilled water in a mixer-grinder for 5 minutes. The resulting water extract was then filtered using filter paper and utilized for the analysis of chemical composition, mineral content, antioxidant properties, total Sinigrin and Glucosinolates.

Fig. 1. *Brassica nigra* plant and seeds

The prepared extract was stored in the refrigerator and was intended for use within seven days, after which it was discarded. Fresh extracts were prepared for subsequent experiments **(Kokate *et al*., 1995).**

2.2- Determination of chemical composition of mustard seeds

The technique established by the **Association of Official Analytical Chemists (1980)** was utilized to assess the diverse chemical composition of mustard seeds. The analysis was conducted in triplicate, and the resulting data were averaged. Proximate analyses included moisture (%), protein (%), carbohydrate (%), dietary fiber (%), total fat, and ash content (%). The evaluation of tannin content in plant materials was executed following the Folin-Ciocalteu method **(Singleton *et al*., 1999)**.

2.3- Mineral Composition Analysis

The mineral formatting of mustard seeds, which includes macronutrients like calcium, magnesium, sodium, potassium and phosphorus, along with micronutrients like iron, manganese, copper, selenium and zinc, was evaluated using atomic absorption spectroscopy (AAS). Initially, the samples underwent wet digestion with a combination of nitric acid and perchloric acid, in accordance with the AOAC 975.03 method **(AOAC, 2005)**. Subsequent to

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digestion, the samples were evaluated using AAS to determine the concentration of each mineral.

2.4- Antioxidant Properties

2.4-1 Determination of Total Phenol

The total phenol content in the extract was assessed following the method established by **Singleton et al. (1999)**. A volume of 0.2 ml of the extract was combined with 2.5 ml of 10% Folin-Ciocalteu's reagent and 2 ml of 7.5% sodium carbonate. This mixture was incubated at 45 °C for 40 minutes, after which the absorbance was measured at 700 nm using a spectrophotometer, with gallic acid serving as the standard phenol.

2.4-2 Determination of Total Flavonoid

The total flavonoid content in the extract was quantified using a colorimetric assay developed by **Bao et al. (2005)**. Initially, 0.2 ml of the extract was mixed with 0.3 ml of 5% $NaNO_3$. After 5 minutes, 0.6 ml of 10% $AlCl_3$ was added, and after an additional 6 minutes, 2 ml of 1 µM NaOH was introduced to the mixture, followed by 2.1 ml of distilled water. The absorbance was recorded at 510 nm using a spectrophotometer against a reagent blank, and the flavonoid content was expressed as mg rutin equivalent.

2.4-3 Determination of Ferric Ion-Reducing Antioxidant Power (FRAP)

The reducing capacity of the extract was evaluated using the method described by **Pulido et al. (2000)**. A volume of 0.25 ml of the extract was mixed with 0.25 ml of 200 µM sodium phosphate buffer at pH 6.6 and 0.25 ml of 1% KFC. The mixture was incubated at 50°C for 20 minutes, after which 0.25 ml of 10% TCA was added and centrifuged at 200 rpm for 10 minutes. Subsequently, 1 ml of the supernatant was combined with 1 ml of distilled water and 0.1% $FeCl_3$, and the absorbance was recorded at 700 nm.

2.4-4 Determination of Fe^{2+} Chelation

The chelation ability of the extract for Fe^{2+} was assessed using a modified method as described by **Puntel *et al.* (2005).** In brief, 150 µM $FeSO_4$ was added to a reaction mixture containing 168 ml of 0.1 µM Tris-HCl at pH 7.4, 218 ml of saline, and the extract, with the total volume adjusted to 1 ml using distilled water. The reaction mixture was incubated for 5 minutes prior to the addition of 13 ml of 1,10-phenanthroline, after which the absorbance was measured at 510 nm with a spectrophotometer.

2.4-5 Evaluation of Free Radical Scavenging Capacity

The capacity of the extract to scavenge free radicals was evaluated against DPPH (1,1-diphenyl-2-picrylhydrazyl) in accordance with the method established by **Gyamfi *et al.* (1999).** Approximately 1 ml of the extract was combined with 1 ml of a 0.4 µM methanolic DPPH solution, and the resulting mixture was allowed to sit in the dark for 30 minutes before the absorbance was measured at 516 nm using a spectrophotometer.

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2.4-6 Evaluation of Total Antioxidant Activity

This assay is predicated on the reduction of Mo (VI) to Mo (V) by the extract, leading to the formation of a green phosphate/Mo (VI) complex under acidic conditions, as detailed by **Prieto *et al.* (1999)**. A volume of 0.1 ml of various concentrations of the extract was mixed with 3 ml of a reagent solution (comprising 0.6 µM sulfuric acid, 28 µM sodium phosphate, and 4 µM ammonium molybdate). The tubes were subsequently incubated at 95°C for 90 minutes. After cooling the mixture to room temperature, the absorbance was recorded at 695 nm using a spectrophotometer, with the blank serving as a control. The antioxidant activity was expressed in terms of gallic acid equivalents.

2.4-7 Estimation of Sinigrin Content

The assessment of Sinigrin content within the sample was carried out following the methodology outlined by **Kolodziejczyk *et al.* (2019).** Absorbance measurements were recorded at 330 nm, utilizing methanol as the blank.

2.4-8 Estimation of Total Glucosinolates

The spectrophotometric evaluation of methanolic extracts was executed employing the technique established by **Mawlong *et al.* (2017)**, which incorporated sodium tetrachloropalladate. Absorbance was quantified at 425 nm against a blank that was prepared using the identical procedure but excluding the extract.

Experiment 2

2.1- Mustard seed sterilization and germination

Seeds of black mustard (*Brassica nigra*) were procured from the Agricultural Research Centre. Initially, they were rinsed under running tap water for 5 minutes to eliminate solid particles and dust. This was followed by immersion in 70% ethyl alcohol for 1 minute, and subsequently, they were sterilized using 30% commercial Clorox (1.25% sodium hypochlorite) combined with Tween-20 for 10 minutes. The seeds were then washed three times with sterilized distilled water.

The sterilized seeds were germinated on pre-sterilized medical cotton pads that had been moistened with distilled water, all carried out under aseptic conditions within 200 ml glass jars. After one week of culturing the seeds, the fully germinated seedlings were transferred to a solid MS medium supplemented with varying concentrations of Gibberellic acid (GA_3) at 0.1, 0.5, 1.0, 2.0 and 3.0 mg/l, all performed under a laminar flow hood. Subsequently, they were incubated in a culture room for 30 days at a temperature of 25±2°C, following a photoperiodic regime of 16 hours of light and 8 hours of darkness under cool white fluorescent lights (2700-Lux) to facilitate seed germination and the collection of explants. The percentage of seed germination was recorded after 30 days of incubation.

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2.2- Culture Medium:

The culture medium utilized for *in vitro* cultures was the basal nutrient medium known as Murashige and Skoog (MS) **(Murashige and Skoog, 1962)**, which was supplemented with the following additives (mg/l): 0.5 pyridoxine-HCl, 0.5 nicotinic acid, 0.1 thiamine-HCl, 100 myo-inositol, 30 g/l sucrose, and solidified with 6 g/l agar.

The pH of the culture medium was adjusted to 5.8 using either 1.0 µM HCl or 0.5 µM NaOH before autoclaving. Following this, the medium was dispensed into small jars (150 ml) in 30 ml portions per jar and sealed with polypropylene lids. Subsequently, the media were autoclaved for 20 minutes at a pressure of 1.5 kg/cm and a temperature of 121°C.

2.3- Callus induction

For the purpose of callus induction, *in vitro* germinated seedlings that were 25 days old from the time of culturing served as the source of explants.

Segments measuring approximately 1.0-2.0 cm in length were excised from the hypocotyls and cotyledons (3-5 cm with 2 mm petioles) of the seedlings using sterile blades. The five-segment explant pieces were arranged horizontally in the callus induction medium, which was composed of MS medium enriched with different concentrations of 2,4-Dichlorophenoxyacetic acid (2,4-D) at levels of (0.0, 0.1, 0.5, 1.0, and 2.0 mg/l), in addition to a constant concentration of Benzyl amino purine (BA) (0.5 mg/l). These explants were incubated in a dark culture environment at a temperature of 25 ± 2°C for a duration of 2 months, which included two subcultures, to promote callus initiation. Throughout this time, the explants were re-cultured on the same medium every 4 weeks. All treatments were conducted with three replications.

After 8 weeks from the initiation of callus, data were collected regarding the percentage of callus induction and the fresh weight of the callus (g) for each treatment.

2.4- Establishment of Cell Suspension Culture

To establish cell suspension cultures, pieces of friable callus that were eight weeks old, derived from hypocotyl and cotyledon cultures, were transferred to a liquid medium. Each jar contained approximately 1.0 g of calli, with each glass jar holding 30 ml of MS liquid medium supplemented with 1.0 mg/l 2,4-D and 0.5 mg/l BA, and incubated for two months on a horizontal shaker (120 rpm) at 25±2ºC in complete darkness. The control treatment was cultured in a liquid MS medium that was free of supplements.

During this period, every 15 days, the callus fragments were aseptically filtered through Whitman No.1 filter paper to eliminate larger aggregates. Subsequently, 10 ml of the filtrate was combined with 30 ml of fresh medium, replacing the previous suspension for subculturing. The mixture was then placed on a horizontal shaker at 120 rpm and maintained at a temperature of 25±2ºC in complete darkness. Ultimately, the filtrate underwent centrifugation at 1500 g for 4 hours. The supernatant was further filtered using Whitman No.2, and the resulting extracts

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were collected in a vial and stored at -20°C to produce the final water extract for subsequent applications.

- **Experiment 3**

2.1- Evaluations of the severity of cucumber wilt and root rot disease.

Fusarium wilt and root rot incidence and the severity percentages of cucumber wilt and root rot were evaluated 60 days after planting in greenhouse conditions. The assessment of wilt and root rot was conducted through a visual inspection of the rot symptoms present on the plants. This evaluation method was based on the approach described by **Mahmoud and Abdel Fattah (2020)**.

The infected plants were classified into five groups, each comprising five infected plants, and each group was labelled. The control treatment included plants that remained uninfected. The plants received regular watering twice a week. Each set of five plants served as a replicate.

A numerical scale from 0 to 5 was employed to quantify the severity of root rot **(Aegerter *et al*., 2000)**, based on the type and severity of the observed symptoms. The severity scale was defined as follows:

0: No symptoms detected.

1: Initial symptoms manifest on the plant, usually on the first leaf (affecting 10% of the root), with mild secondary root rot.

2: Symptoms are visible above the true leaf, with 25% of the root system exhibiting browning.

3: The entire plant displays signs of wilting, with 50% of the root system dark brown, showing clear root rot symptoms and dead secondary roots.

4: The plant shows complete wilting, with 76–100% of the roots dark brown and extensive root rot symptoms.

5: The entire plant has perished.

2.2- Isolation and Identification of the Causes of Cucumber Wilt and Root Rot.

Approximately 20 infected roots were collected from cucumber plants displaying symptoms of wilt and root rot within a greenhouse during the 2024 growing season. These specimens were transported to the Plant Pathology Department at the National Research Centre in Dokki, Egypt.

The symptoms observed included yellowing and ulceration of the leaves, root decay, and brown discoloration of the roots. To facilitate further analysis, isolation tests were conducted on the collected samples. The affected roots were meticulously washed with tap water, air-dried, and then cut into small pieces to initiate the isolation process. A 0.5% sodium hypochlorite solution was employed for surface sterilization of these root segments for a

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duration of three minutes. Following this, the sterilized root pieces were rinsed multiple times with sterilized distilled water and then dried between sterile filter sheets. Subsequently, three sections of each sterilized root segment were placed onto Petri dishes containing Potato Dextrose Agar (PDA) medium. The Petri dishes were incubated at a temperature of 25±2°C for 5-7 days, with periodic inspections conducted. Once the Fusarium mycelial growth had developed sufficiently, it was transferred to fresh PDA plates utilizing the hyphal tip method **(Brown, 1924)** and cultured for an additional seven days.

The resulting pure cultures were stored at 4°C. The isolated fungi were identified as *Fusarium solani* and *Fusarium oxysporum,* based on the morphological characteristics of the fungal hyphae and conidia, as outlined by **Moni *et al*. (2016)**.

2.3- Effect of Mustard extract on fungal growth *in vitro*

To assess the impact of *in vitro* mustard extract on fungal proliferation, the extract was prepared from suspension cultures at varying doses of 2, 4 and 8 ml/l. In this context, the aqueous extract of mustard was subjected to sterilization via a Millipore filter and subsequently added to the warmed PDA medium (40-45°C) at concentrations of 2, 4, and 8 ml/l. The treated and untreated (control) PDA medium in each conical flask was gently shaken and then dispensed in a consistent volume (15 ml) into sterilized Petri dishes (9 cm in diameter) to allow for solidification. The medium lacking extracts functioned as the control. Mycelium discs, measuring 5 mm in diameter, were extracted from the tips of 7-day-old cultures of the two fungi under investigation using a cork borer, and these discs were then positioned at the center of the Petri dishes. Three plates were allocated for each specific concentration. The plates were incubated at a temperature of 25 ± 2°C. The experiment concluded when the mycelial mats completely covered the surface of the medium in the control treatment. The linear growth (mm) and percentage of growth reduction were calculated after nine days of incubation, following the formula outlined by **Wang *et al*. (2003)**.

2.4- Inoculant preparation and soil inoculation

The experiment was carried out in greenhouse conditions at the Mohamed Naguib Air Base, located on El Alamein Road, Egypt, during the growing seasons of 2024 and 2025. This study specifically focused on cucumber plants, 8 weeks old (cv. Barracuda), which had been infected with Fusarium.

The Mustard inoculant was formulated using a steam-sterilized sand-mustard medium in a 1:3 ratio **(Abdel Moneim, 1996)**. For the inoculant preparation, a mixture of 25 g of clean sand, 75 g of mustard seeds, 2 g of sucrose, 0.1 g of yeast, and 100 ml of water was combined in a 1:3 ratio and placed into 500 ml glass bottles. These bottles underwent steam sterilization at 121°C for 20 minutes over two separate days and were subsequently incubated at room temperature (25 ± 2°C) for a duration of 14 days.

In a greenhouse maintained at approximately 25–30°C with 70% relative humidity, the infected plants were categorized into three groups, each containing five infected plants, and

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each group was distinctly labelled. The control treatment comprised infected plants that did not receive the inoculant. The plants were irrigated regularly, twice a week. Each set of five plants served as a replicate.

The prepared inoculum was added to the soil at rates of 0.5 g, 1.0 g and 2.0 g per plant, ensuring thorough mixing of the inoculum into the soil, followed by irrigation as described by **Mahmoud and Abdalla (2021)**.

Also, we used Teko 8% WDG to control the *Fusarium* sp. The active ingredient is Fosetyl-Aluminium 80% Wettable Powder. The definition was a preventive and curative fungicide for root rot in vegetable plant. It has the ability to move to all parts of the treated plants through the plant sap, and is therefore present in all tissues of the treated plants. Methods of use the compound, spray the plants with Teko compound at a rate of 250 g/100 liters of water once. Treat the soil in the nursery by making a solution of 10 g of Teko compound per 5 liters of water.

The colonies of *F. solani* and *F. oxysporum* were counted and the reduction percentage of fungal growth was recorded after 9 and 15 days from inoculating the soil of sand-mustard medium in soil.

To further explore the impact of the mustard inoculant on the growth of *Fusarium* sp. Scanning Electron Microscope (SEM) was utilized to examine the morphological alterations in fungal mycelia growth with different doses of mustard inoculant.

Nine soil samples (approximately 1 kg) were collected from the soil that grew cucumber plants in it and was then infected with *Fusarium* sp. The soil was sifted to remove excess plant residues. The fungal propagules were quantified as fungal colonies (colony-forming units, CFU) within the soil samples, represented as the number of colony-forming units per gram of air-dried soil (CFU/g soil).

The calculation of CFU/g was performed using the following formula:

$$\text{CFU/g} = \frac{\text{Number of colonies} \times \text{dilution factor}}{\text{Volume of culture plate}}$$

The data were recorded under greenhouse conditions to study the effect of the sand-mustard medium on *Fusarium* sp of cucumber plants, including survival, disease severity score, disease reduction (%), productivity and fruit quality.

2.5- Statistical Analysis

The study utilized a fully randomized block design as outlined by **Gomez and Gomez (1984)**. The data were assessed using analysis of variance through the MSTAT computer program **(MSTAT Development Team, 1989)**. The leading three results from each treatment were subjected to statistical evaluation. Duncan's Multiple Range Test **(Duncan, 1955)** was applied to determine significant differences among the means.

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3. Result and Discussion

Experiment 1

3.1- Determination of chemical composition of mustard seeds

The result of nutrient content, mineral compositions, antioxidant activity and total glucosinolates, sinigrin content profile of the black mustard seed are shown in the following tables:

3.1-1 nutrient content

The nutrient profile of mustard seeds was examined. The findings are presented in **Table (1)**. Nutritional data were recorded per 100 g.

The moisture content of mustard seeds was found to be 5.4 g, a characteristic typical of seeds, which implies good storage stability. The protein content was notably high at 28.0 g, highlighting mustard seeds as an exceptional source of plant-derived protein. The fiber level in mustard seeds was measured at 13.0 g. The fat content was particularly elevated at 26.0 g, suggesting that mustard seeds are also a significant source of beneficial fats. The ash content, which represents the total mineral content, was recorded at 4.7 g, indicating the presence of essential minerals. The carbohydrate content was 36.0 g, contributing an additional source of energy. The tannin content was noted to be 8.7 g/100 g.

Table (1). Nutrients and contents of mustard seeds extract.

Component (g/100 g)	*Brassica nigra* Seeds
Moisture	5.4
Protein	28
Dietary fiber	13.0
Total fat	26.0
Ash	4.7
Carbohydrates	36.0
Tannin	8.7

The mustard plant is notably abundant in essential amino acids and protein content. The protein constitutes 25-30%, rendering it an excellent food source utilized as oil for industrial and commercial applications **(Santos and Galceran, 2002)**. Mustard seeds are composed of carbohydrates, protein, fat and dietary fibre. In addition, they are abundant in vitamins (such as vitamin K and C) and a range of trace minerals (including Fe, Ca, Se, Zn, Mn, Cu and Mg)

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along with electrolytes (K and Na) **(Jaiswal *et al.*, 2012)**. An examination of a seed sample showed the following components: protein, 20; moisture, 8.5; fiber, 1.8; fat, 39.7; carbohydrates, 23.8; minerals, 4.2 as phosphorus, 700; iron, 17.9; calcium, 490; thiamin, 0.65; niacin, 4.0 mg/100 g; riboflavin, 0.26 and carotene, 152/100 g. Moreover, the seeds also contain choline (211 mg/100 g) and ascorbigen **(Stepien *et al.*, 2017)**. Tertiary and tetraterpene alkaloids have been identified in the aqueous extracts derived from the seeds and roots. The seeds exhibit reduced levels of moisture (4.51%), ash (3.22%), and total carbohydrates (8.23%), while presenting elevated levels of protein (23.11%), fat (51.60%), and crude fiber (9.34%) **(Aletor and Adegoke, 2018)**. Brassica plants are known to contain a wealth of nutritionally beneficial components (carbohydrates, lipids, protein, vitamins, and minerals) as well as phytochemically beneficial compounds (glucosinolates, isothiocyanates, flavonoids, and phenolics) **(Paul *et al.*, 2019)**. The seeds exhibit lower percentages of ash (0.23%), moisture (1.26%), and crude fiber (1.80%), while demonstrating higher percentages of crude protein (2.58%) and crude lipid (3.85%) **(Ogidi *et al.*, 2019)**.

Mustard seeds are small, round seeds measuring 1 to 2 mm in diameter, varying in color from yellowish white to black. These seeds contain 24.6 g of protein, 6.2 g of water, 28.4 g of total carbohydrates, 35.5 g of fat, 8.8 g of fiber and 5.3 g of ash. In traditional medicine, Indian mustard is utilized as a remedy for arthritis and foot pain **(Gupta *et al.*, 2021)**. The analysis of proximate composition reveals that mustard seeds serve as a rich source of fat and protein, thereby constituting a significant dietary element for plant-based nutrition. The notable protein content (28.4%) underscores the potential of mustard seeds as a competitive alternative to other plant-derived protein sources **(Singh, 2023)**. In samples of both white and black mustard, proteins and carbohydrates are the predominant compounds, with fats following. Consistent with expectations, the highest fat content was observed in rapeseed samples, reaching up to 33.23% **(Zorica *et al.*, 2023)**.

The tannin concentration was found to be highest (7.75 ± 0.06%) in mustard seeds. This bioactive compound accelerates the healing of wounds and inflamed tissues **(Farquar, 1996)**. Tannins are recognized for their potential in cancer prevention and the treatment of inflamed or ulcerated tissues **(Aleto and Adeogun, 1995)**. The findings of this study align with those reported by other researchers in the same domain **(Sunita *et al.*, 2017)**. A variety of phytoconstituents from the seeds of *B. juncea* were identified through quantitative analysis. The data indicate the presence of qualitative phytochemical components in brown mustard seed, including tannins, terpenoids, saponins, flavonoids, alkaloids, phenols and glycosides **(Ogidi *et al.*, 2019)**. The bioactive compounds present in Brassica seeds, such as polyphenols, proteins, alkaloids, glucosinolates (GLS), carotenoids and fatty acids contribute to various pharmacological properties of medicinal significance, including antimicrobial, anticancer, antioxidant, nematocidal activities and insecticidal **(Singh and Singh, 2025)**.

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3.1-2 Mineral compositions

The findings from the mineral analysis are presented in **Table (2)**. The analysis indicated that mustard seeds possess a comparatively higher concentration of potassium (K), magnesium (Mg), calcium (Ca), sodium (Na), and phosphorus (P), along with a minimal amount of iron (Fe), which are classified as macro-elements and are necessary in substantial quantities. Conversely, the levels of copper (Cu) and manganese (Mn), which are microelements required in lesser amounts, are relatively lower. These results align well with the findings of **Farrell (1990)**. Furthermore, copper (Cu) and manganese (Mn) serve as essential cofactors for antioxidant enzymes.

Table (2). The mineral compositions of mustard seeds extract.

Mineral Content (mg/100 g)	
Macronutrients	**Micronutrients**
Potassium (K) - 736 mg	Iron - 8.7 mg
Magnesium - 380 mg	Manganese - 2.6 mg
Calcium - 299 mg	Copper (Cu) - 0.58 mg
Sodium - 27 mg	Zinc - 5.3 mg
Phosphorus - 925 mg	

Mustard seed is a small, round grain recognized for its various advantages due to the minerals it possesses. Minerals are crucial inorganic nutrients that significantly contribute to the structure, metabolism and overall development of plant cells.

One of the key minerals present in mustard seed is calcium; it constitutes approximately eight percent of the total mineral composition of mustard seed. Minerals such as calcium (Ca) are essential for preserving the integrity of cell walls and the stability of membranes. Calcium fortifies the cell walls by facilitating the formation of calcium pectate, which enables plant cells to withstand mechanical stresses. Calcium ions (Ca^{2+}) function as secondary messengers in signal transduction pathways, assisting plant cells in responding to environmental factors like drought or pathogen invasion **(Jie *et al*., 2015).** In addition to calcium, mustard seeds are rich in several other vital minerals, including phosphorus, magnesium, zinc, potassium, copper, manganese and iron, albeit in lower concentrations.

Magnesium serves as a cofactor for enzymes, enhancing the rate of biochemical reactions. Magnesium (Mg) is a central element in chlorophyll and is indispensable for photosynthesis. It also activates numerous enzymes that are involved in energy metabolism **(Chen *et al*., 2018)**. Meanwhile, phosphorus (P) is essential for normal metabolism, energy production, and cellular growth. Phosphorus is a fundamental component of ATP (adenosine

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triphosphate), the cell's energy currency, and plays a role in energy transfer reactions. It also constitutes part of nucleic acids (DNA and RNA), which are critical for genetic information and protein synthesis **(López-Arredondo *et al.*, 2014)**. Potassium (K) is instrumental in regulating osmotic pressure, which governs water movement across cell membranes. This regulation is vital for maintaining cell turgor pressure, which is essential for keeping plants upright and promoting growth **(Johnson *et al.*, 2022)**. Zinc functions as an antioxidant and is crucial for cellular activities. It is essential for the growth, reproduction, and signaling in plants. Additionally, zinc serves as a cofactor for various enzymes **(Mousavi *et al.*, 2018)**. Minerals such as iron (Fe) and manganese (Mn) participate in electron transport chains located in chloroplasts and mitochondria, playing a significant role in respiration and photosynthesis processes **(Fraser and Chapple, 2011)**. In the context of plants, copper (Cu) is a vital cofactor for numerous proteins that are integral to plant cell metabolism. These proteins are involved in functions such as electron transport in mitochondria and chloroplasts, regulation of the cellular redox state, ethylene perception, and modification of the cell wall. Furthermore, manganese collaborates with copper and iron to facilitate energy production in cells, protein synthesis, and the metabolism of various fats and carbohydrates **(Gong *et al.*, 2021)**.

Mustard leaves are abundant in chlorophyll, ascorbic acid, ß-carotene, calcium, potassium, and a variety of other minerals **(Lim *et al.*, 2000)**. The mineral analysis underscores the nutritional significance of mustard seeds, revealing their high concentrations of magnesium and potassium, which are vital for numerous physiological functions. Furthermore, the inclusion of iron and calcium boosts the nutritional profile of mustard seeds, rendering them a crucial component of a balanced diet **(Singh, 2023)**.

3.1-3 Antioxidant Activity

The antioxidant characteristics of mustard seed extracts were assessed using the DPPH radical scavenging assay. The results are presented in **Table (3)**.

Table (3). Polyphenol and flavonoid compositions and antioxidant activity in Mustard seeds extract.

Antioxidant compositions	
Total Phenolic Content	6.5 mg/g
Total Flavonoid Content	2.8 mg/g
FRAP	42.6 %
Fe^{2+}	34.3 %
DPPH	65.2 %
Total Antioxidants	75.2 %

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The antioxidant properties in **Table (3)** show the presence of phenolics, flavonoids, ferric ion reducing antioxidant (FRAP), Fe $^{2+}$ chelator, 1,1-diphenyl-2-picryhydrazyl (DPPH), and total antioxidants. The result showed total phenolics (6.5 mg/g), flavonoids (2.8 mg/g), FRAP (42.6%), Fe^{2+}(43.3%), and the DPPH free radical scavenging test (65.2%), indicated that mustard seeds demonstrated significant antioxidant activity, achieving an inhibition rate of 75.2%. It was found that mustard seeds possess the highest level of DPPH, while flavonoids show the lowest level. Rich in phenolic compounds, mustard seeds enhance their antioxidant capabilities. These bioactive compounds may help reduce oxidative stress and decrease the risk of various diseases.

In recent years, there has been a significant surge of interest in phenolic compounds owing to their potent antioxidant properties. The antioxidant efficacy of these phenolics is primarily linked to their redox characteristics, which enable them to function as hydrogen donors, reducing agents, metal chelators, and free radical scavengers. The appeal of phenolic compounds can be ascribed to their bioavailability, bioactivity, and antioxidant capabilities **(Lobo *et al*., 2010)**. Additionally, it is conceivable that the phytochemicals found in black mustard seeds may work synergistically to manifest the aforementioned antioxidant properties **(Wu *et al.,* 2004).**

The flavonoid concentration, quantified at 2.48%, indicates that the plant samples exhibit biological functions, including antioxidant activity, as well as defense against inflammation, allergies, platelet aggregation, free radicals, viruses, and microbes **(Okwu, 2004)**. Polyphenols constitute a class of phenolic compounds distinguished by their multiple hydroxyl groups and are widely distributed across various plant species. They act as crucial secondary metabolites in plants and are primarily located in the leaves, skins, shells, roots, and fruits. The majority of polyphenolic compounds are hydrophilic substances found within cells and are predominantly present in glycosidic form after bonding with carbohydrates. Polyphenols include tannins and flavonoids, both of which have exhibited significant antioxidant activity **(Cartea *et al.,* 2011)**. The total phenol content of the methanol extract was quantified at 171.73 gallic acid equivalents, while the total flavonoid content was recorded at 7.45 quercetin equivalents. The principal phenolic compound identified through HPTLC was gallic acid, with ferulic acid, quercetin, rutin, and caffeic acid following in prominence **(Rajamurugan *et al*., 2012)**.

Furthermore, FRAP (Ferric ion reducing antioxidant power) assays have demonstrated the antioxidant potential of mustard and its polyphenolic constituents. This activity exhibited a linear correlation with flavonoid content, suggesting that the antioxidant effects may be influenced by the levels of flavonoids and polyphenols **(Kim *et al*., 2016)**. Mustard is abundant in vitamin C, vitamin A, glucosinolates, phenolic compounds and various other substances that exhibit antioxidant characteristics **(Park *et al*., 2017)**. The antioxidant efficacy of a 50% acetonitrile extract from mustard was evaluated, revealing that the antioxidant activity of mustard seeds was marginally superior to that of other parts.

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Reports indicate that the total phenolic compound content in mustard varies from 3.3 to 404.3 mg of gallic acid equivalent/g. Additionally, research has established that mustard is particularly rich in flavonoids, with *Brassica juncea* containing flavonoid levels ranging from 4.0 to 395.3 mg quercetin equivalents/g **(Sun *et al.*, 2018)**. An examination of the phytochemical profile has revealed that mustard seeds are rich in phenolic compounds and flavonoids, which are both acknowledged for their strong antioxidant properties. The notable total phenolic content (65.3 mg GAE/g) and total flavonoid content (28.7 mg QE/g) suggest that mustard seeds may improve the antioxidant capabilities of plants. The significant antioxidant activity of mustard seeds, evidenced by the DPPH inhibition assay (82.5%), highlights their potential function in protecting against oxidative stress and associated diseases. This antioxidant capability may assist in alleviating damage inflicted by free radicals, thus lowering the risk of various diseases **(Singh, 2023)**. The total polyphenol content in the samples analyzed varied between 5.5 and 11.1 mg GAE/g DM. White mustard exhibited a significantly higher polyphenol content, whereas black mustard had the lowest polyphenol content **(Zorica *et al.*, 2023)**. The polyphenol content in different sections of the plant can be ranked as follows: seed > leaf > root > stem **(Kim *et al.*, 2016)**. Furthermore, research has indicated that mustard is abundant in flavonoids.

Numerous antioxidant compounds have been identified in mustard **(Park *et al.*, 2017)**, which include phenolic compounds, vitamin A, glucosinolates, vitamin C, and various other substances.

The antioxidant activity of the methanol extract from *Brassica nigra* seeds and leaves was demonstrated across a broad concentration range of 10-500 µg/ ml, with activity increasing as concentration rose **(Rajamurugan *et al.*, 2012)**. The scavenging activities of antioxidants for DPPH are linked to their ability to donate hydrogen **(Rahman *et al.*, 2015)**. Polyphenols and their subclass flavonoids, which are well-established as potent antioxidants, are present in plant extracts **(Karak, 2019)**. Flavonoids were among the primary components found in the ethanol extract and are likely significant contributors to the antioxidant activity.

The antioxidant properties of mustard seeds enhance their applications in the food industry and healthy management. To evaluate the antioxidant potential, mustard seeds were assessed for DPPH activity and total antioxidant activity. The seeds exhibited approximately 50% inhibition at a concentration of 3.0 mg of dry seeds. Similarly, **Kumari *et al.* (2016)** also reported high antioxidant activity in mustard. Total polyphenolic compounds demonstrate a strong positive correlation with total flavonoids. The content of total polyphenolic compounds showed a moderate positive correlation with DPPH ($r = 0.524$), whereas a significantly high correlation was observed between total flavonoid content and DPPH assays ($r = 0.906$). Given that the antioxidant activity is attributed to the content of polyphenol compounds, it can be concluded that the high antioxidant properties in the analyzed oil crops primarily stem from the flavonoids present in the samples. This is due to the well-established high radical scavenging potential of these compounds. Furthermore, flavonoids are recognized as the predominant polyphenolic compounds in the majority of oilseeds as well **(Abiodun, 2017)**.

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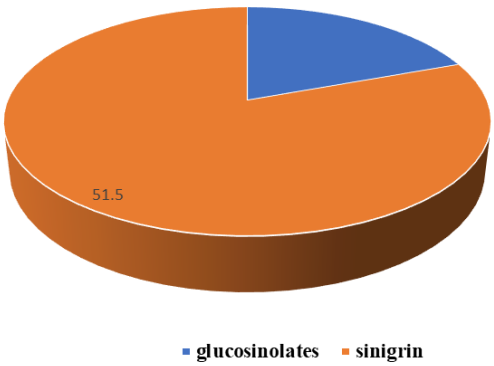
3.1-4 Determination of total Glucosinolates and Sinigrin Content

The biochemical of mustard seeds was done in the current study. The study revealed the presence of anti-nutrient compounds **(Fig. 2)** like Sinigrin content in Mustard was 51.6 mg/g and glucosinolates content in Mustard was 12.4 mg/g.

Fig. 2. Total of Glucosinolates and Sinigrin content mg/g



The phytochemicals present in mustard, as analyzed, align with the relevant research conducted by **Janhavi *et al.* (2022)**. *Brassica nigra*, commonly known as black mustard, is extensively cultivated for its seeds, which exhibit a blackish brown-red hue and possess a slight bitterness. The predominant glucosinolate found in the seeds of black mustard is sinigrin, which can be hydrolyzed to form allyl-isothiocyanate (AITC), contributing to its distinctive pungent and irritating odor, as noted by **Divakaran and Babu (2016)**. According to **Avita (2013)**, Brassica is known to contain a significant concentration of glucosinolates, which, upon enzymatic digestion, yield cytotoxic compounds exhibiting antifungal properties. Compounds such as dimethyl disulfide, carbon disulfide, methanethiol and dimethylsulfide, which are produced during the degradation of glucosinolates, may play a crucial role in the suppression of fungal growth.

Glucosinolates are secondary metabolites of plants that contain sulfur and are hydrophilic in nature **(Mitreiter and Gigolashvili, 2021)**. These compounds are synthesized in various quantities and forms across numerous plant tissues and organs.

Among the anti-nutritional compounds discussed herein, *Brassica nigra* exhibits the highest levels of total glucosinolate and sinigrin, which are identified as the most significant components **(Lietzow, 2021)**. The levels of glucosinolates and their degradation products in mustard plants, particularly in the seeds, are primarily influenced by the activity of myrosinase **(Mithen, 2001)**. The seeds generate a greater quantity of glucosinolates in comparison to other plant parts such as leaves, stems, and flowers **(Zrybko *et al.*, 1997)**. The characteristics and concentration of glucosinolates in mustard vary based on species and distribution, including sinalbin (0.1%–1.1%) found in yellow mustard, sinigrin (0.8%–0.9%) in Oriental mustard, and (0.4%–0.9%) in black mustard **(Bell *et al.*, 2018)**.

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Sinigrin is an important glucosinolate present in the seeds of brown mustard (B. juncea) and black mustard (B. nigra), serving as the primary compound that converts into allyl-isothiocyanate (AITC) following hydrolysis. In contrast, the predominant glucosinolate in white mustard (S. alba) is sinalbin, which yields 4-hydroxybenzyl isothiocyanate **(Nguyen *et al.*, 2020). Kim *et al.* (2016)** reported that the total glucosinolate content in mustard was quantified at 13.0 mg/g. Sinigrin was identified in all mustard varieties, with concentrations reaching up to 53.8 mg/g. **Nugrahedi *et al.* (2013)** indicated that in fresh mustard, sinigrin constituted over 90% of the overall glucosinolate content.

Mustard exhibiting strong pesticide activity contains a sinigrin concentration of approximately 2.5% by weight **(Tsao *et al.*, 2002)**. Quantitative analysis revealed that seed extracts had the highest sinigrin content, averaging 12.75 µg/l, which was significantly different from other plant parts. Furthermore, no significant differences were observed between true leaves, cotyledon leaves and stem extracts, which showed sinigrin values of 7.0, 7.1 and 6.8 µg/g, respectively **(Al Shahawany *et al.*, 2016)**. The findings of the present research agreement with those of **(Oh *et al.*, 2015)**, who determined that the seeds of *B. juncea* possess a greater concentration of sinigrin compared to other parts.

Experiment 2

3.2- Mustard seed sterilization and germination

Soaking the seeds in 70% ethyl alcohol for 1 minute, followed by sterilization with 30% commercial Clorox combined with Tween-20 for 10 minutes, has been identified as the most effective method for surface sterilization, yielding the lowest contamination rates and achieving an average seed germination percentage of 83.7%.

The previous authors achieved that before conducting any *in vitro* experiments, it is essential to ensure the proper sterilization of both the explant and the media. The effectiveness of the sterilization process is influenced by two primary factors: the type of disinfectant used and the duration of exposure of the explant to the disinfectant **(Gantait and Kundu, 2017)**. Elevated concentrations of sodium hypochlorite have been shown to enhance the germination of *Brassica nigra* seeds, potentially due to a scarification effect on the seed coat that facilitates better water and oxygen absorption, or as a result of increased oxidative respiration through the generation of additional oxygen from the breakdown of sodium hypochlorite **(Yildiz and Celal, 2002)**. The sterilizing solution comprises 6.2% sodium hypochlorite, a potent disinfectant that is commonly employed for surface sterilization in plant cell and tissue culture studies **(Derso and Feyissa, 2015)**. The viability of seeds diminishes if the sterilization duration exceeds 2 minutes for 70% ethyl alcohol and 15 minutes for 30% sodium hypochlorite. Consequently, both seed contamination and germination rates are influenced by these disinfectants **(Kumar *et al.*, 2017)**.

An additional procedure suggested by certain researchers has been incorporated into the seed sterilization protocol, which entails treating the seeds with a 0.1% (w/v) mercuric chloride solution for 5 minutes subsequent to the application of 70% alcohol and a 20% sodium

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hypochlorite solution. The concluding washing step is executed with sterile distilled water multiple times to eliminate any residual mercuric chloride, as it poses a risk to the explants **(Qamar *et al.*, 2014)**. Similarly, seeds were sterilized for 2 minutes by immersing them in 50% ethanol, followed by a wash in 50% (v/v) sodium hypochlorite, and finalized with a rinse using sterile distilled water **(Gerszberg *et al.*, 2015)**.

In general, *in vitro* plantlets of Brassica are recognized to arise from seeds. The seeds were effectively sterilized by rinsing them with a 1% (v/v) sodium hypochlorite solution, which was enhanced with minor quantities of Tween-20 detergent; subsequently, the seeds were immersed in a 70% ethanol solution and ultimately washed several times with sterile distilled water **(Ravanfar *et al.*, 2015)**. The seeds were subjected to treatment with 0.1% mercuric chloride (w/v) for 2–3 minutes, followed by extensive rinsing with sterilized distilled water. Four seeds were placed into each flask. The flasks were maintained in a growth chamber at 25°C in darkness. The germination rate was assessed on the fifth day post-inoculation of the seeds **(Zafar *et al.*, 2016)**.

In this study mature seeds grown on MS medium supplemented with GA_3 at various concentrations (0.1, 0.5, 1.0 2.0 and 3.0 mg/l) has been applied to mustard seeds to determine the effect on germination as shown in **Table (4) and Fig. (3)**. Increasing the concentration of GA_3 increased the germination rate, followed by a decrease. The average germination percentage showed the highest percentage (82.5%) at 1.0 mg/l GA_3 concentration, and the decrease occurred when the concentration of GA_3 increased. While the lowest percentage (10.2%) was recorded in the control seeds.

Table (4). Effect of various concentrations of GA_3 on the germination of Mustard Seeds after 30 days of incubation.

GA_3 concentration (mg/l)	Germination %
Control	10.2 f
0.1	26.6 de
0.5	43.7 c
1.0	82.5 a
2.0	50.3 b
3.0	33.0 d

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Fig. 3. Mustard seedling germinated on MS medium containing 1.0 mg/l GA_3

Brassica seeds commenced germination after a period of 7 days when cultured on MS medium. The fully developed seedlings, exhibiting two leaf stages, emerged 20 days post-germination of the seeds **(Munir *et al*., 2008)**. **Sundhari (2014)** indicated that gibberellins are involved in processes such as cell division, stem elongation, leaf expansion, and an increase in the total number of leaves. **Sumampow (2002)** noted in his research that at a concentration of 200 ppm of gibberellins, the cultivated plants demonstrated optimal results in terms of both shoot and root length. **Sitangang *et al.* (2015)** emphasized the necessity of administering gibberellins at the appropriate dosage. An excess of gibberellins in the medium can become toxic, thereby hindering plant growth. Conversely, inadequate concentrations have minimal impact on seed development. According to **Arianto *et al.* (2018)**, elevated levels of gibberellins significantly influence the height of germinating shoots and the length of roots in nutmeg seeds.

The enhancement in germination percentage observed with GA_3 may be ascribed to its function in activating cellular enzymes, along with the increased pliability of the GA_3 cell wall and its superior water absorption capabilities **(Padma *et al*., 2013)**. The rise in germination percentage among GA_3-treated seeds can be explained by the role of GA_3 in breaking seed dormancy, which facilitates earlier and more effective seed germination through the release of substances like auxin and gibberellin **(Singh *et al*., 2016)**.

3.3- Callus induction

After 8 weeks from the callus initiation from hypocotyls and cotyledon explants, data were recorded as the callus induction percentage and callus fresh weight (g) were recorded for each treatment.

Under suitable and specific conditions of the nutrient medium and the types and concentrations of plant growth regulators, callus formation is expected from any vegetative parts derived from any living part of the plant. However, the rate of callus formation varied depending on the type and quantities of plant growth regulators, as well as the samples used.

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Cotyledons and hypocotyls from laboratory-grown seedlings of uniform size (1 cm) were used as samples in this study.

The explants initially showed swelling, followed by the formation of callus within two weeks of incubation, except for the control treatment. The callus development began at the cut tips of cotyledons and hypocotyls on MS medium supplemented with varying concentrations of 2,4-D (0.0, 0.1, 0.5, 1.0 and 2.0 mg/l), along with 0.5 mg/l of BA, and extended throughout all samples. Callus formation from the cut edges of each plant sample commenced after 7 days in cotyledon samples and after 15 days in hypocotyls samples. The callus induced from the cotyledon samples exhibited the most rapid growth during the initial 7–25 days of cultivation. After about 5 weeks of cultivation, the explants had completely converted into callus.

Table (5). Impact of various concentrations of 2,4-D with 0.5 mg/l BA on callus induction from cotyledons and hypocotyls after 8 weeks of culture.

2,4-D concentrations (mg/l)	Callus induction (%)		F.W. (g)	
	Cotyledons	Hypocotyls	Cotyledons	Hypocotyls
Control	0.0 e	0.0 d	0.0 d	0.0 d
0.1	25.0 d	10.0 c	0.6 c	0.3 c
0.5	41.4 c	23.4 b	1.0 b	0.7 b
1.0	84.6 a	40.5 a	2.3 a	1.5 a
2.0	50.0 b	20.6 b	0.8 bc	0.6 b

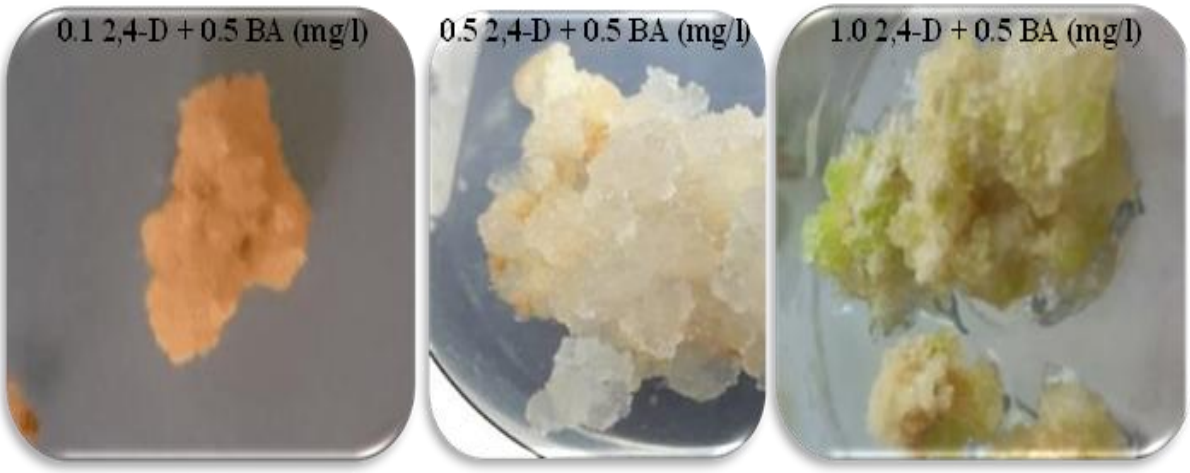

Fig. (4). The callus induction from Cotyledons on MS medium containing different concentrations of 2,4-D after 8 weeks of culture.

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Fig. (5). The callus induction from Hypocotyls on MS medium containing different concentrations of 2,4-D after 8 weeks of culture.

Call formation was observed in 25.0% to 84.6% of the cotyledon samples in MS medium supplemented with varying concentrations of 2,4-D, **Fig (4)**. Callus formation was also observed in 10.0% to 40.5% of the hypocotyls samples **Fig. (5)**. In the cotyledons, callus formation began with a light creamy white color and a firm texture. The highest response to *in vitro* callus stimulation (84.6%) and the highest fresh callus weight (2.3 g) were recorded in MS medium supplemented with 1.0 mg/l of 2,4-D and 0.5 mg/l of BA. However, when samples of hypocotyls were used to stimulate callus formation, the response was delayed, resulting in very little callus formation. The control treatment didn't induced any callus on explants as shown in **Table (5)**.

The type and amount of callus, as well as the efficiency of callogenesis, were influenced by the duration of hormone exposure and the specific kind of explant used. In this study, the greatest amount of callus was observed on cotyledons following a short induction period on MS medium with 2,4-D. Similar findings have been documented in other studies; **Ullah *et al.* (2004)** noted that in *Brassica napus* explants, callus growth commenced at the cut ends of both hypocotyls and cotyledons. **Abdellatef and Khalafallah (2008)** reported similar results in *Brassica napus* cultivated on MS media supplemented with 2,4-D and BA. A notable variation in the rate of callus formation was detected among various explants, plant growth regulators, and genotypes. Conversely, **Al-Naggar *et al.* (2008)** presented contrasting results for Brassica, indicating that the compound 2,4-D is recognized as one of the most commonly utilized auxins for *in vitro* callus induction across a range of plant species. 2,4-D facilitated callus formation across the entire surface of cotyledons and hypocotyls.

Consistent with the current findings, **Osman *et al.* (2010)** suggested that the optimal hormone concentration is contingent upon the genotype employed and the type of explants used. A comparison of callus initiation from hypocotyl and cotyledon explants revealed that cotyledon explants exhibited a higher percentage of callus initiation at 2,4-D concentrations compared to hypocotyl explants. The ideal concentration of 2,4-D for initiating callus from

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cotyledon explants was determined to be 0.5 mg/l, which resulted in the highest callus initiation and yielded calli with a greater average weight relative to other concentration levels in *B. napus* **(Ali *et al.*, 2007)**.

Regarding the influence of varying concentrations of BA on callus initiation from hypocotyl and cotyledon tissues, it was found that 0.5 mg/l BA was more effective in inducing callus from cotyledon explants than from hypocotyls. Conversely, a higher concentration of BA (1.5 mg/l) was observed to impede callus proliferation. Lower concentrations of BA were demonstrated to be more effective for callus induction in *Brassica napus* **(Mollika *et al.*, 2011).**

Tan *et al.* (2010) indicated that the combination of 2,4-D and kinetin in MS medium led to the greatest callus weight. Conversely, **Khater *et al.* (2013)** found that explants of *Atropa belladonna* cultivated on media with 2,4-D produced the highest callus weight from leaf explants. In contrast, **Alagumanian *et al.* (2014)** reported that *Tylophora indica* attained the maximum dry weight from nodal explants at a concentration of 5 mg/l of 2,4-D, whereas the minimum was observed at 0.5 mg/l. **Dubey and Gupta (2014)** effectively induced callus formation from cotyledon samples grown on a medium containing 0.5 mg/l of 2,4-D in conjunction with 0.5 mg/l of NAA. Additionally, **Lone *et al.* (2016)** noted the highest callus induction when utilizing 2,4-D within the concentration range of 2.0-2.5 mg/l.

A superior *in vitro* response for callus induction from cultured mature cotyledons was observed on MS medium supplemented with 3.0 mg/l of 2,4-D **(Shyam *et al.*, 2021)**. Our findings indicated that a concentration of 1.0 mg/l of 2,4-D along with 0.5 mg/l of BA resulted in the highest rate of callus induction, corroborating previous studies that demonstrated the synergistic effects of auxins and cytokinins on callus formation in mustard **(Ćosić *et al.*, 2023)**.

3.4- Establishment of cell Suspension Culture

To produce an embryonic cell suspension culture, approximately 1.0 g of embryonic callus from cotyledon and hypocotyl explants, which were well-formed, fragile and white in color, was transferred to liquid MS medium supplemented with 1.0 mg/l of 2,4-D and 0.5 mg/ of BA. During shaking, the delicate callus easily shattered and separated into clusters varying in size from 2.0 to 5.0 mm. Additionally, shaking further broke these clusters into smaller cell aggregates. After 60 days of cultivation, the mustard extract was collected and utilized as an inhibitor against *Fusarium spp*.

Plant cell culture demonstrates significant potential for the *in vitro* synthesis of complex secondary metabolites **(Nasim *et al.*, 2010)**. The secondary metabolites derived from plants serve various functions, including as food additives, agrochemicals, flavors, fragrances, colorants and bio-pesticides **(D'amelia *et al.*, 2021)**. Depending on their synthesis methods, secondary metabolites are typically categorized into three primary groups: terpenes, alkaloids and phenols **(Movahedi *et al.*, 2021)**.

The production of valuable secondary metabolites occurs through plant cell suspension culture. To initiate cell suspension cultures, friable calli are typically introduced into a liquid

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medium within flasks, which are subsequently placed on a rotating shaker. A cell suspension culture is established as the newly generated cells disperse throughout the liquid medium. After a period of two to three weeks, the suspended cells are transferred to fresh media, while the larger fragments are discarded. In comparison to cells in callus culture, those in suspension often exhibit significantly enhanced rates of cell division. Therefore, when rapid and large-scale cell production is required, cell suspension represents the most efficient method **(Chandran *et al.*, 2020)**. Rather than utilizing whole plants, suspension cultures can generate valuable compounds under controlled conditions that remain unaffected by variations in soil or climate. Cultured cells can easily proliferate to produce the specific metabolites of any plant cell, and automated controls can be implemented to augment the production of secondary metabolites and manage cell proliferation **(Vijaya *et al.*, 2010)**. Plant cell and tissue culture are crucial in promoting the accumulation of secondary metabolites in medicinal and aromatic plants. Research has indicated *in vitro* callus induction and enhanced production of secondary metabolites in *Convolvulus alsinoides* **(Kaladhar, 2012)** and *Solanum trilobatum* **(Priya and Chellaram, 2014)**. The combination treatments of 2,4-D with BA have proven to be more effective in inducing callus formation compared to other combinations of NAA with BA, particularly at lower concentrations of 2,4-D (0.5 and 1.0 mg/l). This highlights the efficacy of 2,4-D in facilitating cell growth and division. The influence of 2,4-D on calli formation has also been investigated in other medicinal plants, such as *Achyranthes aspera* **(Sen *et al.*, 2014)** and *Glinus lotoides* **(Teshome and Feyissa, 2015)**. The relationship between 2,4-D and BA has demonstrated notable impacts on the rates of callus formation in comparison to the interaction of NAA and BA concentrations. These results are consistent with many other research studies conducted on different plant species **(Padmavathy, 2014)**.

Commercially important plant compounds are secondary metabolites, classified into three categories: glycosides, essential oils and alkaloids **(Chiocchio *et al.*, 2021)**. Essential oils consist of a mixture of terpenoids used as fragrances, flavoring agents and solvents. Glycosides include phenols, flavonoids, saponins, tannins, mustard oils and cyanogenic glycosides, which serve as pharmaceuticals and pigments and coloring agents for foods (e.g., antibiotics, steroid hormones and digitalis) **(Sajc *et al.*, 2000)**.

Recently, there has been a growing interest in utilizing plant cell suspension culture for the production of high value secondary metabolites derived from plants that hold commercial significance **(Rischer *et al.*, 2022)**. Numerous factors have been identified as influencing the accumulation of secondary metabolites in stem cells and suspension cells. The recent research has indicated that the nutrient composition and carbon source of the culture medium play a necessary role in metabolic processes, leading to the accumulation of both biomass and secondary metabolites **(Ghosh *et al.*, 2018)**. The most critical factors include the chemical composition of the medium used as growth enhancers; in this context, the incorporation of paracetamol and angiotensin-converting enzyme inhibitors into tissue culture media has a notable effect on the distribution and *in vitro* produced of secondary metabolites in *Stevia rebaudiana* **(Radic *et al.*, 2016)**.

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The callus derived from hypocotyl explants of *Brassica nigra* was analyzed for its anti-bacterial and antioxidant characteristics against four pathogenic bacterial strains: *Staphylococcus aureus*, *E. coli*, *K. pneumonia* and *Ps. Aureogenosa*. It was generally observed that both antibacterial and antioxidant activities were more significant in calli cultivated under light incubation conditions compared to those grown in darkness or in the original plant tissues from which the calli were derived. Furthermore, it was noted that older calli exhibited improved antioxidant activity, elevated levels of total phenolics and more potent antibacterial effects **(Hussein *et al.*, 2010)**.

- **Experiment 3**

3.5- Different symptoms associated with Fusarium wilt disease in cucumbers.

Research on pathogenicity revealed that both species of Fusarium significantly impacted the growth of cucumber plants. The control plants exhibited no symptoms and stayed healthy. Cucumber plants infected with *Fusarium solani* and *Fusarium oxysporum* displayed comparable disease symptoms, including leaf yellowing and wilting. The yellowing symptoms first appear in the older leaves, which subsequently turn yellow and wilt **(Fig. 6)**.

Fig. 6. Different symptoms associated with Fusarium wilt disease in cucumbers.

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The **Table (6)** summarizes the data on disease incidence, root rot severity, and symptoms. The results revealed that the disease incidence in the greenhouse ranged from 22.0% to 75.8%. However, the disease was also observed on young plants with low infection rates. Fusarium caused the highest root rot severity, reaching 18.5% and 85.7%, respectively.

Table (6). Survey of *Fusarium* spp. on cucumber (cv. Barracuda) under greenhouse conditions during growing season.

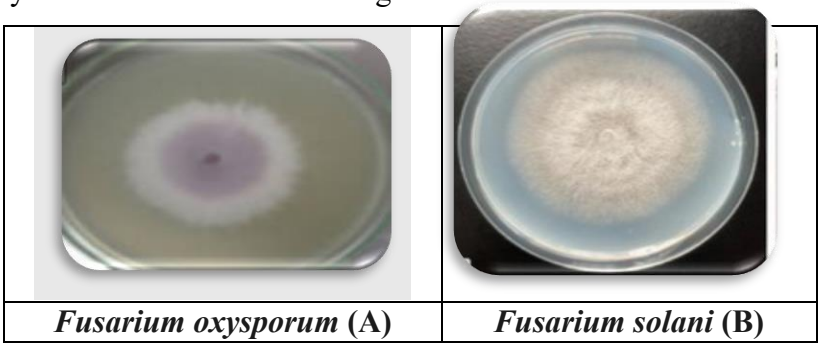
Symptoms	Disease incidence (%)	Disease severity %
Wilt, leaf yellowing	**22.0 e**	**18.5 de**
Wilt	**75.8 a**	**85.7 a**
Wilt, leaf yellowing	**53.5 b**	**64.8 b**
Wilt	**42.3 c**	**54.5 c**
Wilt	**30.7 d**	**20.2 d**

These results agreement with the observations by **Chehri *et al.* (2011)**, who noted that various *Fusarium* species, such as *F. proliferatum, F. oxysporum, F. semitectum F. equiseti* and *F. solani*, were responsible for root and stem rot in cucurbits. **Ibrahim and Al-Juboory (2024)** identified *Fusarium* species as the primary agents responsible for root rot in cucumber.

3.6- Isolation and identification of cucumber Fusarium wilt and root rot.

Pathogenic organisms were isolated by growing them on a PDA medium at a temperature of 25°C ± 2 for duration of 7 days. Through morphological analysis, two species of Fusarium were recognized. The isolates were identified as *F. oxysporum* f.sp. *cucumerinum* and *F. solani*.

The morphological examination of *F. oxysporum* colonies **(Fig. 7)**, showed a dense white cotton-like mycelium accompanied by a dark-purple underside on PDA. As for *F. solani* produced exhibits a dense growth of white, creamy mycelium within its colony **(Fig. 7)**, along with soft, woolly hairs and an abundance of growth.



***Fusarium oxysporum* (A)** | ***Fusarium solani* (B)**

Fig. 7. The cultural characteristics of *Fusarium oxysporum* (A) and *Fusarium solani* (B) growth on potato dextrose agar (PDA).

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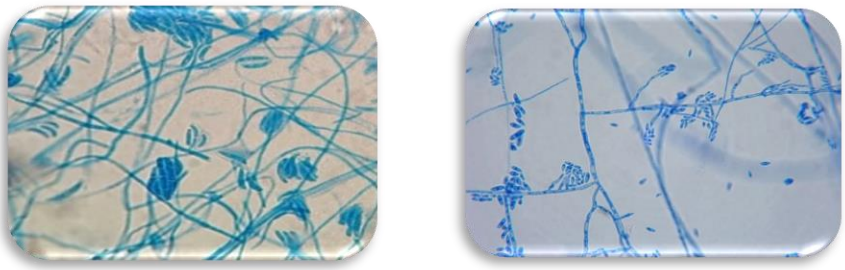
Microscopic conidospores

The macro-conidia of *Fusarium oxysporum* were oval and tapering, with cell walls segmented into multiple cells (typically 3-5 cells). The macroconidia measured approximately 15.6 µm in length and 4.2 µm in width, with the number of septa varying from 2 to 3. Micro conidia displayed forms that ranged from oval to kidney-shaped. as shown in **Table (7)**. Chlamydospores were produced both individually and in chains. These chlamydospores are globose and have rough walls **(Fig. 8)**.

As for *Fusarium solani* the macro conidia measured approximately 35.6µm in length and 6.2 µm in width, with the number of septate varied from 3 to 5 septate **(Table 7 and Fig. 8)**. In contrast, there numerous microconidia were present, displaying an oval or kidney-shaped form. Chlamydospores were noted to develop either singly or in pairs. The sizes of the microconidia varied from 13.3 µm in length and 5.4 µm in width.

Table (7). The morphological characters of isolated *Fusarium oxysporum* and *F. solani*

Isolate code	Conidia measurement (µm)				Number of septate
	Micro-conidia		Macro-conidia		
	Length	Width	Length	Width	
FOX	8.3 b	5.4 a	15.6 b	4.2 b	2–3 b
FS	13.3 a	5.4 a	35.6 a	6.2 a	3–5 a



Conidiospores of *F. solani* **Conidiospores of *F. oxysporum***

Fig. 8. The morphological characteristics of *Fusarium solani* and *Fusarium oxysporum* grown on PDA media.

The results of this study indicate that the *F. solani* isolates produced a considerable quantity of aerial mycelium, exhibiting colony colors that ranged from white to light purple. These traits are consistent with the observations made by **Sonkar *et al.* (2014).** Furthermore, the morphological and microscopic characteristics of *F. oxysporum* were in agreement with

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those described by **Ibrahim *et al.* (2015)**, who noted that the pigmentation of *F. oxysporum* on potato dextrose agar (PDA) varied from white to purple, with microconidia displaying a tapered apical cell and a foot-shaped basal cell. The microconidia were characterized as oval and kidney-shaped, containing 0–1 septa.

Fusarium oxysporum is recognized as a major pathogen affecting a wide range of plants worldwide. **Din *et al.* (2020)** documented the presence of Fusarium wilt disease in cucumbers in Peninsular Malaysia, linked to *Fusarium oxysporum* and *F. solani.*

Fusarium species were classified according to their morphological characteristics **(Suresh *et al.*, 2019)**. The morphological traits of *Fusarium* species play a vital role in the early stages of distinguishing between different Fusarium species **(Din *et al.*, 2020)**.

Fusarium species produce a range of secondary metabolites, such as mycotoxins and extracellular cell wall-degrading enzymes (CWDEs), which facilitate the weakening and successful invasion of the host plant **(Perincherry *et al.*, 2021).**

Fusarium oxysporum is recognized as a significant pathogen impacting numerous plants globally, as noted by **Din *et al.* (2020)**, who documented the incidence of Fusarium wilt disease in cucumbers in Peninsular Malaysia, associated with *Fusarium oxysporum and F. solani.*

The current study indicates that the symptoms of Fusarium wilt in affected cucumbers initiated with the yellowing of leaves, particularly the older ones, which subsequently spread to the upper parts of the plants. Eventually, the entire plant began to wilt, leading to considerable wilting. These observations align with earlier studies **(Singh *et al.*, 2021).**

Abu Bakar *et al.* (2013) found that the pigmentation of *F. oxysporum* on PDA ranged from white to purple, with micro-conidia displaying a tapered apical cell and a foot-shaped basal cell. The micro-conidia were noted to be oval and kidney-shaped, with 0–1 septate. The results indicated that *F. oxysporum* exhibited variations in its cultural and morphological characteristics, which corroborate the findings reported by **Sonkar *et al.* (2014)**.

3.7. Impact of mustard extract on mycelial growth of *Fusarium solani* and *Fusarium oxysporum in vitro*

After incubating the plates of Fusarium for nine days at 25 ± 2°C, the *in vitro* mustard extract from suspension cultures at different doses of 2, 4 and 8 ml/L was used against *Fusarium* sp.

Data presented in **Table (8)** and **Fig. (9)** demonstrated that *in vitro* mustard extract treatments reduced the growth of mycelial and increased the growth reduction % of *Fusarium solani* compared to the control. The highest increase in growth reduction percentage (83.3%) were observed at 8.0 ml/l mustard extract concentration, followed by 4.0 ml/l concentration and the lowest increase in growth reduction (34.3%) at concentration 2.0 ml/l compared with control treatment.

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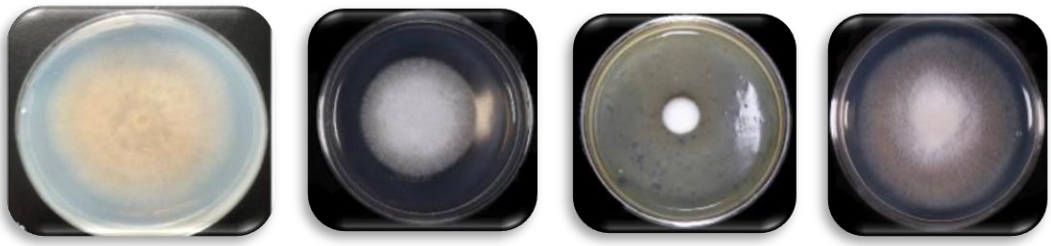
Table (8). Impact of mustard extract on mycelial growth of *Fusarium solani in vitro*

Treatment	Concentration ml/l	Mycelial growth (cm)	Growth reduction (%)
mustard extract	2.0	6.7 b	34.3 c
	4.0	5.2 c	42.2 b
	8.0	1.5 d	83.3 a
Control		9.0 a	0.0 d



2.0 ml /l mustard extract **4.0 ml/l mustard extract** **8.0 ml/l mustard extract** **Control**

Fig. (9). Impact of mustard extract on mycelial growth of *Fusarium solani in vitro.*

Also, data presented in **Table (9)** and **Fig. (10)** demonstrate that *in vitro* mustard extract treatments reduced the growth of mycelial and increased the growth reduction % of *Fusarium oxysporum* compared to the control. The highest increase in growth reduction percentage (75.6%) were observed at 8.0 ml/l mustard extract concentration, followed by 4.0 ml/l concentration and the lowest increase in growth reduction (30.0%) at concentration 2.0 ml/l compared with control treatment.

Table (9). Impact of mustard extract on mycelial growth of *Fusarium oxysporum in vitro*.

Treatment	Concentration ml/l	Mycelial growth (cm)	Growth reduction (%)
mustard extract	2.0	6.3 b	30.0 c
	4.0	5.0 c	44.4 b
	8.0	2.2 d	75.6 a
Control		9.0 a	0.0 d

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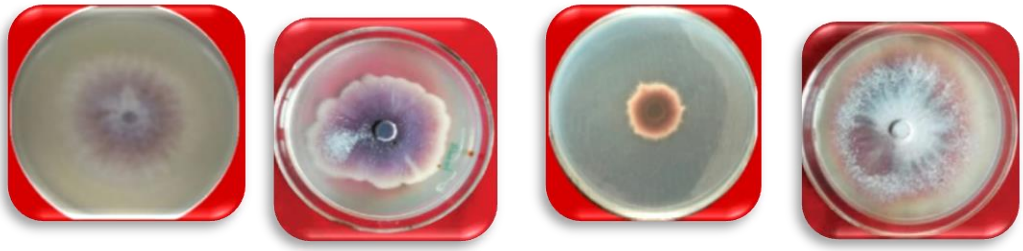

2.0 ml/l of mustard extract | **4.0 ml/l of mustard extract** | **8.0 ml/l of mustard extract** | **Control**

Fig 10. Impact of mustard extract on mycelial growth of *Fusarium oxysporum in vitro.*

According to **Fierro *et al.* (2013)**, the resistance of *F. oxysporum* to commercially available fungicides, along with the frequent application of synthetic fungicide treatments, necessitates the creation of innovative management strategies.

The Brassicaceae family is well-known for its antimicrobial properties. All extracts derived from *Brassica rapa* displayed dose-dependent antifungal effects at various concentrations. The extract from the roots demonstrated inhibition percentages greater than 45% within the concentration range of 10 to 0.1 g/l. Additionally, extracts from the stem, leaves, and seeds also showed significant enzyme inhibition (> 30% and > 35%, respectively) at the same concentration levels. In a study conducted by **Rongai *et al.* (2015)**, fourteen plant extracts were identified as effective in inhibiting the radial growth of *F. oxysporum* f.sp. *lycopersici* when compared to the untreated control. Moreover, extracts from six different plant families (Brassicaceae, Alliaceae, Lamiaceae, Lythraceae, Verbenaceae and Solanaceae) were also found to exhibit notable antifungal activity against *F. oxysporum* sp. *lycopersici*, completely preventing conidial germination.

The increasing interest in the use of mustard plants for various non-food applications is noteworthy. For example, the seed meal of yellow mustard (*S. alba*) has proven effective in weed control, while the seed meal of oriental mustard (*B. juncea*) has been utilized as a broad-spectrum pesticide to manage nematodes, insects, and fungi **(Kayaçetin, 2020)**. Additionally, the toxicity of the purified fraction from black Brassica (*Brassica nigra*) seeds highlights its significant potential as a molluscicide, effectively killing snails at very low concentrations (50–5.96 mg/l within 96 hours) in comparison to both synthetic and plant-derived molluscicides **(Singh *et al.*, 2024)**.

4. Inoculant Preparation and Soil Inoculation

4.1. Effect of Bio-fumigation and Teko 80% WDG fungicide on Pathogenic Fungal Growth

Adding the prepared inoculum of sand-mustard medium to the soil at a rate of 0.5 g, 1.0 g and 2.0 g/plant, thoroughly mixing the inoculum into the soil, and irrigating the soil.

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Effect of sand-mustard medium on pathogen propagules, the results presented in **Table (10)** show that the soil containing cucumber plants infected with *F. solani*, which was treated with a sand-mustard medium at all treatments reduced the count of fungus after 9 and 15 days of inoculation and also reduced the reduction percentage of fungal growth compared to uninoculated soil (control treatment). Data indicate that uninoculated soil showed the highest count of fungus (9.0×10^6 spores/g of soil), and there was not any reduction in the percentage of fungi growth, compared to the soil treated by sand-mustard medium at 2.0 g/plant which recorded the lowest count of fungus (3.3×10^6 and 1.0×10^6 spores/g of soil) and the least reduction in the percentage of fungi growth (57.3 and 28.2%), respectively after 9 and 15 days of inoculation.

However, the Teko 80% WDG fungicide treatment resulted in the fungi content of *F. solani* in soil was 7.0 × 106 and 6.0 × 106 spore/g of soil and recorded the reduction percentage of fungal growth of 86.2 and 80%, respectively after 9 and 15 days of inoculation.

Table (10). Impact of treated soil by sand-mustard medium and Teko 80% WDG fungicide on propagules of *Fusarium solani* in soil after 9 and 15 days of incubation.

Sand-mustard medium							
Control		**0.5 g/plant**		**1.0 g/plant**		**2.0 g/plant**	
Count of fungus (spores/g of soil)							
9 days	**15 days**	**9 days**	**15 days**	**9 days**	**15 days**	**9 days**	**15 days**
9.0×10^6 a	15.0×10^6 **a**	6.4×10^6 b	4.7×10^6 **b**	5.2×10^6 c	2.3×10^6 **c**	3.3×10^6 d	1.0×10^6 **d**
Reduction (%)							
Control		0.5 g/plant		1.0 g/plant		2.0 g/plant	
9 days	**15 days**	**9 days**	**15 days**	**9 days**	**15 days**	**9 days**	**15 days**
0.0 d	0.0 **d**	85.3 a	64.2 **a**	74.0 b	52.6 **b**	57.3 c	28.2 **c**
Teko 80%							
Count of fungus (colony/g of soil)				**Reduction (%)**			
9 days		**15 days**		**9 days**		**15 days**	
7.0×10^6 a		6.0×10^6 ab		86.2 a		80.5 ab	

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To further explore the impact of the mustard inoculant on the growth of *Fusarium solani*. SEM was utilized to examine the morphological alterations in fungal mycelia growth with different doses of mustard inoculant, **Fig. 11**.

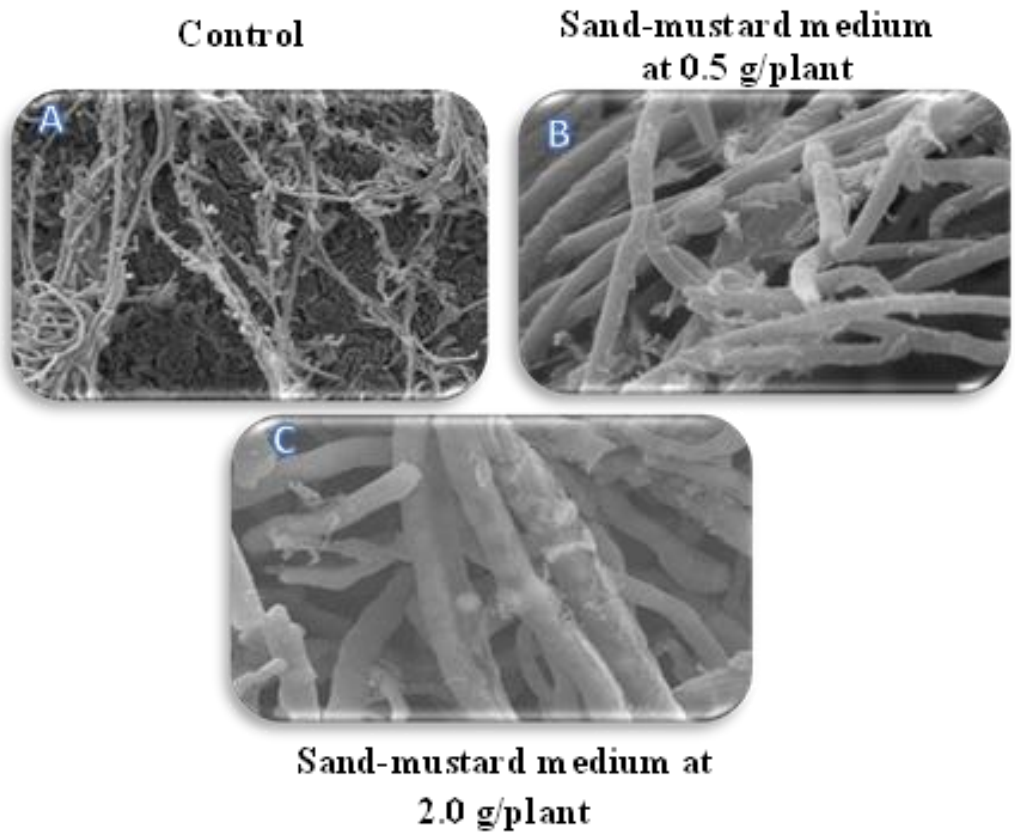

Fig. 11. Scanning electron micrographs of *Fusarium solani* mycelia. (A): Control, (B): Treatment with sand-mustard medium at 0.5 g/plant (C) Treatment with sand-mustard medium at 2.0 g/plant.

Scanning electron micrographs of *Fusarium solani* revealed that different doses of sand-mustard medium were influencing the morphology of *F. solani* mycelia. The control mycelia appeared plump and smooth, regular, well-defined edges and a smooth surface **(Fig. 11)**. Following treatment with sand-mustard medium at 0.5 g/plant resulted in the hyphae lost their smooth appearance, becoming distorted and fractured. While at sand-mustard medium at 2.0 g/plant, the mycelium appeared broken and thicker, lacking conidia. These results suggest that sand-mustard medium impacts the morphology and compromises the structure of *F. solani* mycelia, which is likely responsible for the inhibited growth of the mycelia.

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Table (11). Impact of treated soil by sand-mustard medium and Teko 80% WDG fungicide on propagules of *Fusarium oxyspourum* in soil after 9 and 15 days of incubation.

Sand-mustard medium							
Control		**0.5 g/plant**		**1.0 g/plant**		**2.0 g/plant**	
Count of fungus (spores/g of soil)							
9 days	**15 days**	**9 days**	**15 days**	**9 days**	**15 days**	**9 days**	**15 days**
8.0×10^6 a	12.0×10^6 **a**	5.3×10^6 b	4.2×10^6 **b**	4.0×10^6 c	2.5×10^6 **c**	2.8×10^6 d	0.6×10^6 **d**
Reduction (%)							
Control		**0.5 g/plant**		**1.0 g/plant**		**2.0 g/plant**	
9 days	**15 days**	**9 days**	**15 days**	**9 days**	**15 days**	**9 days**	**15 days**
0.0 d	0.0 **d**	73.3 a	60.2 **a**	65.0 b	43.5 **b**	45.2 c	18.0 **c**
Fungicide							
Count of fungus (colony/g of soil)				**Reduction (%)**			
9 days		**15 days**		**9 days**		**15 days**	
7.0×10^6 a		6.0×10^6 ab		86.2 a		80.5 b	

Effect of sand-mustard medium on pathogen propagules: The results presented in **Table (11)** show that the soil containing cucumber plants infected with *F. oxysporum*, which was treated with a sand-mustard medium, decreased the count of fungi in soil as compared to the (uninoculated soil) control after 9 and 15 days of inoculation of mustard medium. The sand-mustard medium at 2.0 g/plant was the most effective treatment and had a significantly reduced count of fungus to 2.8×10^6 and 0.6×10^6 (spores/g of soil), respectively, after 9 and 15 days of inoculation of mustard. Also recorded the least percentage of fungal growth to 45.2 and 18.0%, respectively, after 9 and 15 days of inoculation. Data indicate that uninoculated soil showed the highest count of fungus (8.0×10^6 and 12.0×10^6 spores/g of soil), after 9 and 15 days of inoculation, respectively and there was not any reduction in the percentage of fungi growth.

However, the Teko 80% WDG fungicide treatment resulted in the fungi content of *Fusarium oxyspourum* in soil was 7.0×10^6 and 6.0×10^6 spore/g of soil and recorded the reduction percentage of fungal growth of 86.2 and 80%, respectively after 9 and 15 days of inoculation.

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To further explore the impact of the mustard inoculant on the growth of *Fusarium oxyspourum*. SEM was utilized to examine the morphological alterations in fungal mycelia growth with different doses of mustard inoculant, as shown in **Fig. 12**.

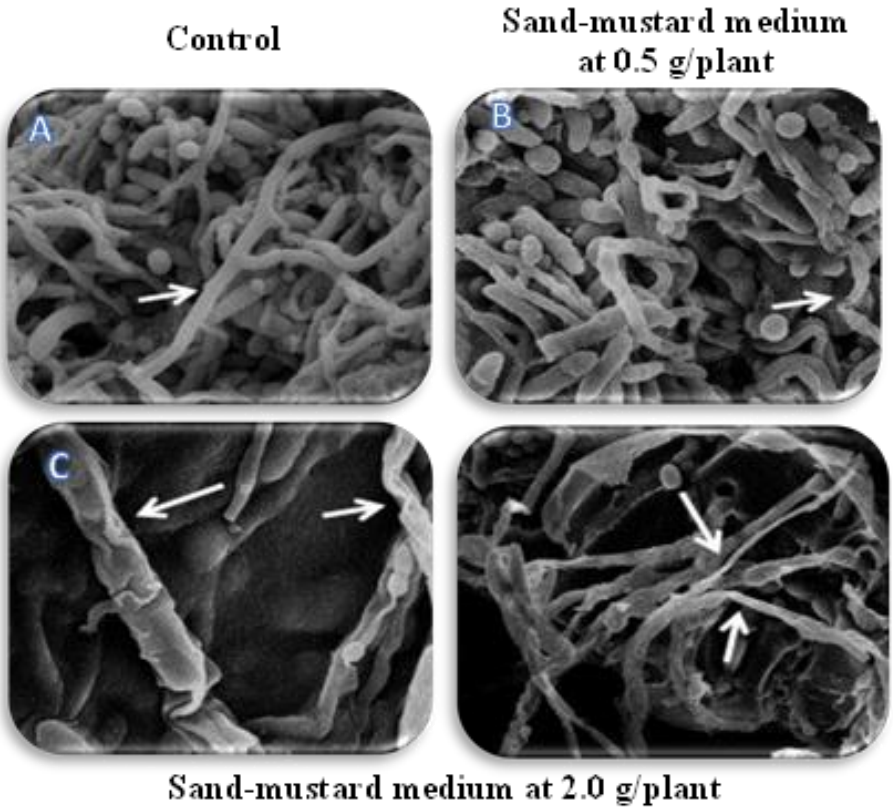

Fig. 12. Scanning electron micrographs of *Fusarium oxyspourum* mycelia. (A): Control, (B): treatment with sand-mustard medium at 0.5 g/plant, (C): Treatment with sand-mustard medium at 2.0 g/plant.

The application of sand-mustard medium significantly changed the morphology of the mycelium and conidia of *F. oxysporum*. In the control treatment, the mycelia were well-formed and continuous **(Fig. 12)**. Across all treatments, the cell wall was thin along the fungus's hyphae. In the treatment with 0.5 g/plant of sand-mustard medium, the mycelium appeared distorted with areas of discontinuity due to hyphal breakage. In the treatment with 2.0 g/plant of sand-mustard medium, the mycelia were dehydrated and uneven, with notable pore-type collapse formation.

From these results indicated that when used the sand-mustard medium as soil inoculation, resulted in the lowest fungi content of *Fusarium* sp. and recorded the reduction percentage of fungal growth rather than used of Teko 80% WDG fungicide.

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4.2- Evaluation of sand-mustard medium and Teko 80% WDG fungicide for controlling Fusarium wilt and root rot disease on cucumber plants *in vivo*.

4.2-1 Impact of sand-mustard medium and Teko 80% WDG fungicide on Fusarium wilt of cucumber plants under greenhouse conditions.

Results in **Table (12)** demonstrate that all concentrations of sand-mustard medium and used Teko 80% WDG fungicide to control infection by *Fusarium oxysporum* were administered both considerably decreased the percentage of infected plants and raised the healthy plants percentage in comparison to the control treatment. In this regard, the treatment at 2.0 g/plant resulted in the lowest percentage of infected plants to 16.7%, and the highest healthy plants percentage to be 83.3%, was followed by the treatment at 1.0 g/plant, which recorded the reduction percentages of infected plants was 46.3% and the healthy plants percentage as 53.7%. On the other hand, the treatment of Brassica seeds powder at 0.5 g/plant was the least effective in healthy plants percentage at 27.2%. The Teko 8% WDG fungicide treatment recorded an infected plants percentage of 43.5% and a healthy plants percentage of 56.5%, respectively.

Table (12). Impact of sand-mustard medium and Teko 80% WDG fungicide on *Fusarium oxysporum* of cucumber plants under greenhouse conditions.

Treatment	Concentration (g/plant)	Survived plants		Disease Severity (%)	Disease reduction (%)
		Infected plants (%)	Healthy plants (%)		
Sand-mustard medium	0.5	72.8 b	27.2 d	7.4 c	89.8 c
	1.0	46.3 c	53.7 c	3.5 d	95.1 ab
	2.0	16.7 e	83.3 a	1.2 e	98.3 a
Teko 80% WDG		43.5 cd	56.5 b	9.2 b	87.4 d
Control		94.7 a	5.3 e	72.8 a	0.0 e

Table (12) shows the effect of different concentrations of sand-mustard medium on the disease severity. The lowest disease severity score was recorded when the infected plants were treated with 2.0 g/plant of sand-mustard medium, which was rated as the most effective compared to the control treatment. The data also reveal that the effect of sand-mustard medium on disease reduction percentage was investigated; the highest percentage (98.3%) was recorded

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with the concentration of mustard seed powder at 2.0 g/plant compared with the control treatment.

However, when used the Teko 80% WDG fungicide treatment resulted in a disease severity score of 9.2% and the disease reduction percentage was 87.4% respectively.

4.2-2 Impact of sand-mustard medium and Teko 80% WDG fungicide on Fusarium root rot (*Fusarium solani*) of cucumber plants under greenhouse conditions.

- Results in **Table (13)** demonstrate that used all concentrations of sand-mustard medium and Teko 80% WDG fungicide to control infection by *Fusarium solani* resulted in decreased the percentage of infected plants and raising the healthy plants percentage in comparison to the control. In this regard, the infected plants treated with 2.0 g/plant mustard seeds powder resulted in the lowest infected plants, 23.7% and the highest percentage of healthy plants, 76.3%, and was followed by the treatment at 1.0 g/plant mustard seeds powder in terms of the reduction percentages of infected plants 53.2% and the healthy plant percentage, 46.8%. On the other hand, the treatment of Brassica seeds powder at 0.5 g/plant was the least effective in Healthy plants percentage to 74.5%.

The Teko 80% WDG fungicide treatment recorded an infected plants percentage of 43.5% and a healthy plants percentage of 56.5%, respectively.

Table (13). Impact of sand-mustard medium and Teko 80% WDG fungicide on Fusarium root rot (*Fusarium solani*) of cucumber plants under greenhouse conditions.

Treatment	Concentration (g/plant)	Survived plants		Disease Severity (%)	Disease reduction (%)
		Infected plants (%)	Healthy plants (%)		
Sand-mustard medium	0.5	74.5 b	25.5 d	10.2 b	85.0 c
	1.0	53.2 c	46.8 c	5.6 d	91.8 b
	2.0	23.7 e	76.3 a	3.3 e	95.2 a
Teko 80% WDG		43.5 d	56.5 b	9.2 c	86.5 c
Control		98.6 a	1.4 e	68.4 a	0.0 d

Table (13) shows impact of different concentrations of sand-mustard medium on the disease severity score. The lowest disease severity score 3.3 % was recorded at 2.0 g/plant of mustard seed powder, which was rated as the most effective, compared to the control treatment. However, the highest disease severity score 10.2% was recorded at 0.5 g/plant of mustard seed

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powder, which was rated as the less effective. The data also reveal that the effect of mustard seed powder on disease reduction (%) was investigated, the highest percentage (95.2%) was recorded with the concentration of mustard seed powder at 2.0 g/plant compared with the control treatment.

However, when used the Teko 80% WDG fungicide treatment resulted in a disease severity score of 9.2 and the disease reduction percentage was 86.5 % respectively.

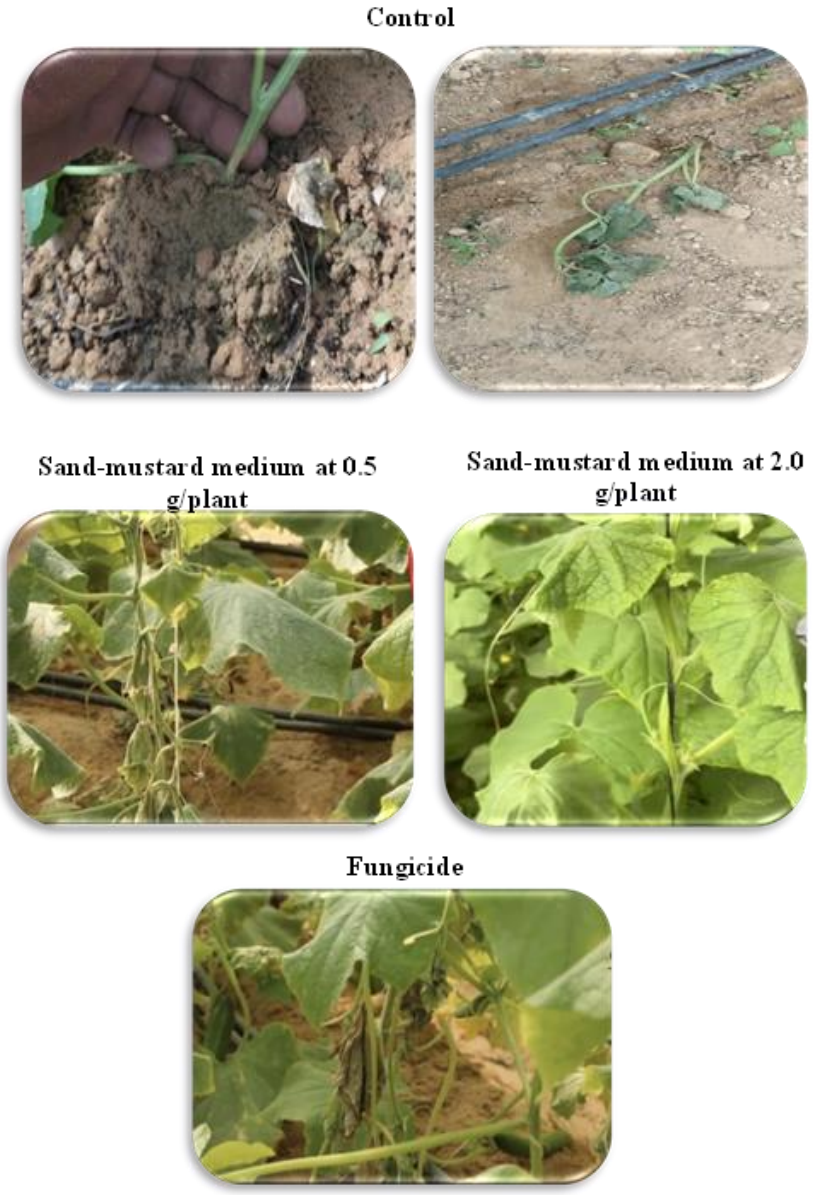

Fig. 13. Impact of different doses of sand-mustard medium and Teko 80% WDG fungicide on *Fusarium* sp. of cucumber plants under greenhouse conditions.

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From these results indicated that when used the sand-mustard medium as soil inoculation, resulted in the lowest infected plants and the highest percentage of healthy plants rather than used Teko 80% WDG fungicide, as shown in **Fig. (13)**.

4.2-3 Productivity and fruit quality

The data in **Table (14) and Fig. (14)** show that the use of sand-mustard medium as a soil inoculant and Teko 80% WDG fungicide resulted in a significant increase in plant height, number of branches, number of leaves, number of fruits and root length of cucumber plants compared to soil not treated with sand-mustard medium (control treatment). The data also showed that the concentration of sand-mustard medium at 0.5 g/plant gave the lowest plant height, number of branches, number of leaves, number of fruits and root length (68.3 cm, 5.4 branches, 24.2 leaf, 13.2 fruit and 15.3 cm/plant, respectively. While the highest values for these parameters (94.0 cm, 10.2 branches, 48.3 leaf, 30.0 fruit and 25.2 cm/plant, respectively) were recorded with the sand-mustard medium treatment at 2.0 g/plant.

However, the treatment of Teko 80% WDG fungicide gave the lowest plant height, number of branches, number of leaves, number of fruits and root length (60.3 cm, 5.2 branches, 21.0 leaf, 15.6 fruit and 14.2 cm/plant, respectively.

Table (14). Effect of sand-mustard medium and Teko 80% WDG fungicide on productivity and fruit quality of cucumber plants under greenhouse conditions

Treatment	Concentration (g/plant)	Plant length (cm)	Number of branches	Number of leaves	Number of fruits/plant	Root length (cm)
Sand-mustard medium	0.5	68.3 c	5.4 c	24.2 c	13.2 cd	15.3 c
	1.0	73.2 b	7.9 b	36.3 b	20.6 b	19.5 b
	2.0	94.0 a	10.2 a	48.3 a	30.0 a	25.2 a
Teko 80% WDG		60.3 d	5.2 c	21.0 cd	15.6 c	14.2 cd
Control		51.5 e	3.3 d	10.0 e	4.5 e	10.4 e

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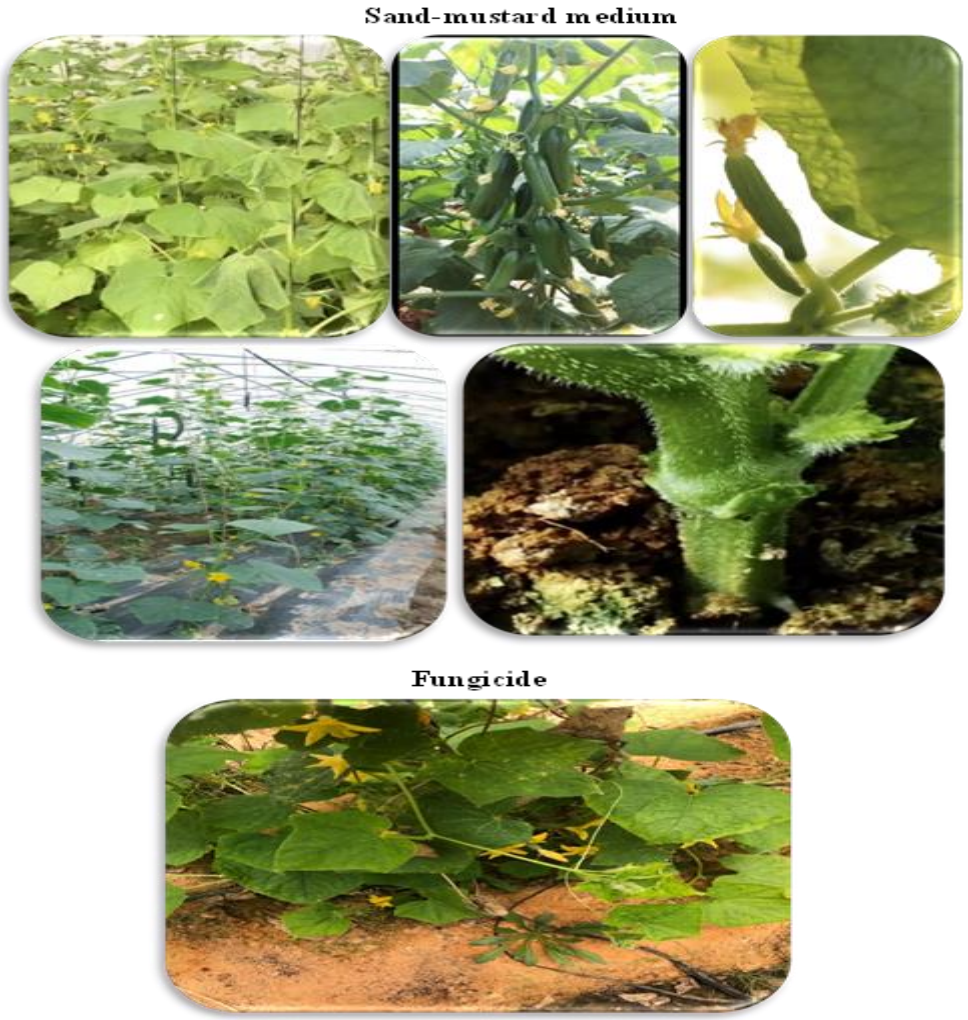

Fig. 14. Impact of sand-mustard medium and Teko 80% WDG fungicide on productivity and fruit quality of cucumber plants under greenhouse conditions.

From these results indicated that when used the sand-mustard medium as soil inoculation, resulted in a significant increase in plant height, number of branches, number of leaves, number of fruits and root length of cucumber plants compared to used Teko 80% WDG fungicide.

The extract of mustard seeds was determined to be the most potent in managing Fusarium wilt in cucumber plants. It significantly diminished the linear growth of the fungus under examination when compared to the control treatment. These findings align with those reported by **(Noble *et al*., 2002)**. Previous studies corroborate these results, including those by **Chung *et al.* (2002)** discovered that the application of a mixture of mustard seed flour (40% ground mustard seeds and 60% Biolan B3 peat) significantly reduced the incidence of seedling wilt induced by Rhizoctonia and promoted the growth of cabbage seedlings. The effect of

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mustard seed flour on the tested fungi may be linked to its biocidal characteristics, particularly the isothiocyanates (ITCs) released during the breakdown of glucosinolates present in mustard seed flour in the soil **(Kirkegaard *et al.*, 2006).**

Mayton *et al.* (1996) reported that the mycelial development of *F. sambucinum* was suppressed by *B. juncea* cultivars with higher concentrations of allyl ITC in soil that had been inoculated with *R. solani*. Additionally, the use of mustard seed meal effectively managed stem infections caused by *R. solani* in lilies **(Os and Lazzeri, 2006)**. The results indicated that applying Brassica green manure at a rate of 25 kg per plot was more effective in controlling root-knot nematodes **(Labrada and Fornasari, 2001)**. **Mazzola and Abi Ghanem (2006)** conducted field trials to assess the effectiveness of Brassicaceae seed meal amendments in managing the complex of soil-borne pathogens and parasites responsible for diseases in apple plants, as well as the potential for these treatments to enhance tree growth and yield.

Furthermore, Indian mustard was found to reduce potato seedling diseases by 40% and 83% **(Robert *et al.*, 2007)**. In this research, we evaluated the capacity of a green manure, specifically Indian mustard, to mitigate Fusarium wilt disease in cucumber plants. The application of Indian mustard manure led to a decrease in the severity of Fusarium wilt disease among cucumber seedlings. Additionally, the green manure (Indian mustard) modified the composition and abundance of bacterial communities present in the rhizosphere of cucumber seedlings. The interactions between plants and microbes in the rhizosphere can significantly affect plant growth and resilience to both biotic and abiotic stresses **(Philippot *et al.*, 2013)**.

Data indicated that soil amendments with mustard seed powder yielded promising outcomes in the management of Fusarium wilt in lupin. The disease was effectively controlled using both mustard seed powder and a fungicide. While Topsin M-70 demonstrated satisfactory results, mustard seed powder proved to be the most effective and reduction the incidence of root rot and wilt disease by 83.6% at both 30 and 90 days post-planting, respectively **(Shaban *et al.*, 2011)**.

Rongai *et al.* (2012) the antifungal properties of 500 botanical extracts were examined against *Fusarium oxysporum* f.sp. lycopersici. The results indicated that around 5.2% displayed an intermediate degree of antifungal activity, whereas merely 3% fully prevented fungal germination. Notably, plants belonging to the Brassicaceae family, known for their high glucosinolate content, generated cytotoxic compounds with antifungal effects after undergoing enzymatic hydrolysis.

The impact of mustard seed powder on the incidence of lupin wilt and root rot was assessed under field conditions throughout the growing season. The results indicate that all treatments tested resulted in a reduction of lupin wilt and root rot incidence, as well as an increase in plant survival rates when compared to the control treatment. Notably, the highest survival rates were observed with the application of mustard seed powder during the growing season, achieving 95.3% and 97%, respectively, in contrast to the control treatment, which recorded 71.3% and 75.6% **(Jin *et al.*, 2019)**. This study examined the effectiveness of organic

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mustard meal as a bio-control agent when used as a seed dressing to combat *Fusarium culmorum*, as well as its impact on the germination potential of wheat *in vitro*, in field conditions, and in a greenhouse. In field trials, applying 15 g of mustard meal along with 45 ml of water for every 1 kg of seed improved the growth and quality of wheat grains **(Kowalska *et al.*, 2021)**.

Growing evidence indicates that alterations in the composition of soil microbial communities can influence plant health and development. The modifications in the microbial communities within the cucumber rhizosphere, resulting from the application of Indian mustard fertilizers, positively affected the growth of cucumber seedlings and their resistance to Fusarium wilt disease **(Zhou *et al.*, 2018)**.

4. Conclusion

The research identified *F. oxysporum* and *F. solani* as the primary pathogens causing damping-off and root rot in cucumbers. Our findings revealed that mustard seeds are abundant in essential mineral nutrients and exhibit high antioxidant activity. Consequently, the study showed that treatments with mustard seed extract significantly inhibited Fusarium growth both *in vitro* and in greenhouse conditions. These treatments also proved effective in mitigating damping-off and root rot in cucumbers and improving cucumber production and fruit quality, offering a highly effective natural disease control method. This approach appears to be a promising strategy for managing Fusarium-related diseases in cucumbers while enhancing plant defense responses.

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Competing Interests: The authors declare that they have no competing interests.

Ethical approval: This manuscript does not contain any studies with human participants or animals performed by any of the authors.

Consent to participate: All authors reviewed and approved the final version for publication.

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