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ANTI-*ASPERGILLUS NIGER* ACTION OF BIOSYNTHESIZED SILICON DIOXIDE NANOPARTICLES ALONE OR COMBINED WITH *MATRICARIA CHAMOMILLA* L. EXTRACT

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ABSTRACT

Fungi are the most frequent microorganisms that cause seed damage throughout development, wreaking much more post- and pre-infections and significantly reducing seed quality. Conventional antifungal agents have failed to overcome a variety of *Aspergillus* spp. These strains have been associated with the development of high-potency mycotoxins, which cause mould infections in fruits and vegetables as well as harmful health effects. Different species, such as *Aspergillus*, *Penicillium*, *Alternaria*, and *Fusarium* were isolated from imported yellow corn samples; however, *Aspergillus* spp. was the most prevalent fungus. The current work attempts to synthesize novel, effective nanomaterials that are stable and antifungal by employing efficient approaches. An extract of *Matricaria chamomilla* L. was used in the biosynthesis of silicon dioxide nanoparticles (SiO_2 NPs) at room temperature. Ultraviolet-visible spectroscopy (UV-Vis), Fourier transform infrared spectroscopy (FT-IR), X-ray diffraction (XRD), transmission electron microscopy (TEM), and Zeta analyses were used to characterize the biosynthesized NPs. The average size of SiO_2 NPs was found to be 17-28 nm. TEM images were used to confirm the biogenesis of spherical-shaped, well-dispersed SiO_2 NPs. The zeta potential graph shows that SiO_2 NPs have a negative potential value (-31.0 mV). The antifungal activity of *M. chamomilla* L. extract, SiO_2 NPs, and SiO_2 combined with the extract was investigated against *A. niger* isolate compared to miconazole. SiO_2 NPs combined with *M. chamomilla* L. extract revealed higher antifungal activity than SiO_2 NPs, *M. chamomilla* L. extract, and miconazole with inhibition zones of 25±0.54, 17±0.37, 20±0.61 and 13±0.23 mm, respectively. This work provides a good alternate technique that is used as an antifungal agent, *M. chamomilla* L. extract supplemented with SiO_2 NPs, against *A. niger*, the pathogen for humans and crop plants.

Keywords: *Matricaria chamomilla*, silicon dioxide, nanoparticles, *Zea mays*, *Aspergillus niger*

INTRODUCTION

Cereal crops are the primary source of nourishment for humans and serve as a supplementary source of nutrition for animals **(Abdel-Nasser et al., 2023)**. Cereals are sensitive to fungal growth, which may be followed by secondary metabolite formation during cultivation, harvest, storage, and processing **(Arroyo-Manzanares et al., 2018)**. Because of the existing climatic circumstances in the Middle East and Africa, such as high temperatures, high humidity, erratic rainfall, and frequent droughts, the proliferation of mycotoxigenic fungus on grains is increasing. Fungi are extensively spread and found anywhere there is moisture, and they are a major source of food and feed spoilage **(Richard, 2007)**. The same occurs for their post-harvest goods, which are frequently subjected to pest attacks, fungal infestations, and/or parasite infection, which can cause major harm to producers and, in certain circumstances, health dangers to consumers **(Faye et al., 2022)**. The dispersion of various fungi in agricultural products has resulted in a reduction in quality and production, as well as considerable economic losses. Maize (*Zea mays* L.) is a significant crop in Egypt. Maize cultivation seeks to provide at least 50% of its output for livestock and poultry feed. In 2023, over 6.5 million metric tons (MMT) of maize were imported since Egyptian maize output is insufficient for human use and is mostly utilized for pasture and animal feed, according to the Foreign Agricultural Service's (FAS) Cairo Forecasts report **(FAS, 2023)**. By importing corn, new pathogens or diseases can spread through seed migrations. Fungi are the most common microorganisms that cause seed damage throughout development, producing more devastation as post- and pre-infections and deteriorating significant seed quality. *Aspergillus niger* is a fungus that is found all over the world and is one of the most prevalent species in the genus *Aspergillus*. *A. niger*, a filamentous ascomycete with fast growth ability, is commonly found as a saprophyte growing on stored grains or seed; it also causes disease on certain fruits and vegetables and is a food toxin. It can produce mycotoxins that are carcinogenic, nephrogenic, and immunological agents **(Jacob & Nair, 2022)**. Mycotoxins are dangerous because of their existence in agricultural and food items; even at low concentrations, they endanger human and animal health by affecting their immune response **(Hussin, 2023)**. One of the most important mycotoxins is ochratoxin, which can cause immune toxicity and carcinogenic, endocrine, chronic, and acute toxicity in humans. *A. ochraceus*, *A. niger*, *Penicillum viridicatum*, and *Penicillum verrucosum* produce ochratoxins **(Janik et al., 2020)**. **Elsamra et al. (2012)** reported the fungal infections in imported corn and studied their negative effects on some nutritional components of stored grains infected with *A. flavus* and *F. verticilloides*. Also, **Abd-Elfatah et al. (2021)** recorded the existence of *Aspergillus* ssp. And their aflatoxins in artificially infested maize grains during storage conditions.

For a long time, the current and indiscriminate use of medications has favoured pathogens in developing resistance to the drugs **(Jacob & Nair, 2022)**. As a result, more appropriate and environmentally friendly medications are discovered to be required. *Matricaria chamomilla* L., also known as chamomile, is a well-known Asteraceae family medicinal herb. It is a cold-resistant annual herb that grows in all soil types **(Srivastava et al., 2010)**. *M. chamomilla* L. is found in southern and eastern Europe, as well as in northern and western Asia. It is now readily available all over the world. *M. chamomilla* L. has traditionally been used to treat a variety of illnesses, including gastrointestinal disorders, the common cold, liver disorders, neuropsychiatric problems, and respiratory problems. This plant is also commonly used to treat pain and infections, as well as skin, eye, and mouth illnesses **(Mihyaoui et al., 2022)**. *M. chamomilla* L. essential oils have a high medicinal value and contain flavones, polysaccharides, and lipophilic active components that correspond to their high biological activities **(Singh et al., 2011)**. Essential oils are mostly used in the aromatherapy sectors, food industry, and perfumery. Still, due to their antiulcerogenic, anti-inflammatory, antiseptic, antimicrobial, sedative, antispasmodic, wound healing, and immunomodulatory characteristics, they are commonly used in the pharmaceutical industry **(Chauhan et al., 2022)**. *M. chamomilla* L. extract was used in the current study, benefiting from all the advantages of its effective active bio-compounds.

However, resistance to pathogens has been a great problem due to the scarcity of antifungal drugs, and some fungi are resistant to various polyherbal formulations that have previously been identified as antifungal agents **(Tan et al., 2022)**. The rising prevalence of fungal infections, the emergence of resistance, and the limits of currently existing antifungal medications highlight the need for the development of novel antifungal drugs, ideally with a different mode of action. As a result, nanobiotechnology was employed as a competitive remedy to this challenge **(El-Zahed et al., 2022a)**. Nanobiotechnology uses biosystems such as plant extracts, fungi, bacteria, and viruses to create cost-effective, safe, and environmentally friendly nanoparticles (NPs). When compared to larger, scale materials, the physicochemical, magnetic, electrical, and biological properties of nanomaterials

with diameters ranging from 1 to 100 nm change dramatically, which makes them more effective than their bulk **materials (El-Fallal et al., 2023; El-Zahed et al., 2022b)**. Silicon dioxide nanoparticles (SiO_2 NPs) were reported to be more effective fungicides against *A. flavus*, *Fusarium oxysporum*, and *A. niger* than bulk SiO_2 **(Tran et al., 2023; Goswami & Mathur, 2022)**. Unfortunately, **Etefagh et al. (2013)** reported the quickly developed resistance of *A. niger* fungi to both NPs and nanolayers. Thus, this study aimed to isolate *A. niger* from *Z. mays* L. and treat it using *M. chamomilla* L. extract alone or combined with SiO_2 NPs as new alternative antifungal agents.

MATERIALS AND METHODS

Isolation, purification, and identification of fungi from imported *Zea mays* L. seeds

Unpackaged cereal grains of yellow corn were purchased from various stores in Damietta governorate, Egypt. Cereal grain samples were transported to the laboratory and kept in polythene bags under refrigeration (4°C). About 10 seeds of *Zea mays* L. were sterilized by putting them into 0.001% mercury chloride solution ($HgCl_2$) for 3 minutes and washed with sterilized distilled water (3 times). Then sterilized grains were blotted dry between sterile Whatman No. 1 filter papers, inoculated into potato dextrose agar (PDA) amended with chloramphenicol (0.1 g/l) plates, and incubated at 30ºC for 5-7 days. After the incubation period, *Aspergillus* spp. growth with black fungal spore appearance was taken and transferred into new PDA agar plates and incubated at 30°C for 3-5 days. Fungal colonies were sub-cultivated on PDA slants stored at 4°C for further use **(Choi et al., 1999)**.

Identification of *Aspergillus* spp. isolates

The fungal colonies were examined and recognized using the morphological, macroscopic, and microscopic criteria based on the keys of **Pitt & Hocking (2009)**, and **Silva et al. (2011)**. The colour of mycelium, reverse colour, size, shape, colour, and surface of conidia, conidiophores, vesicles, hyphae, and mycelia were determined as identification characteristics.

Preparation of *M. chamomilla* L. extract

The *M. chamomilla* L. extract was prepared according to the method described by **Srivastava & Gupta (2009)** with modification. In brief, *M. chamomilla* L. flowers were collected, dried in a shadow area at 25°C, and ground using a grinder machine. Aliquot 5 g from the dried powder was soaked into 50 ml of 70% ethanol (PioChem, Egypt) and put in a shaker (120 rpm, at room temperature; 25°C) for 24 hours. The plant extract was obtained by filtering the suspension using sterile Whatman No. 1 filter papers and then the pure extract was stored at 4°C for further use.

Biosynthesis of SiO_2 NPs

Sodium silicate (Na_2SiO_3) solution was prepared by dissolving 0.0366 g Na_2SiO_3 (purity >98.8%, Sigma-Aldrich) in 100 ml distilled water. 10 ml from the previous solution was mixed with 2 ml NaOH solution at pH 9 (5M, PioChem, Egypt), and then added to 10 ml from *M. chamomile* L. extract. The final mixture was shaken at 120 rpm and 25°C (room temperature) for 3 days. As a result, the mixture was filtered, and the resulting precipitate was thoroughly washed three times with distilled water and methanol. Centrifugation at 8000 rpm for 20 minutes was used to remove the residues from the mixture. The precipitate was then heated in a furnace at 550°C for 45 minutes. The residue was then dried in an oven and stored for future characterization and application operations **(Rahimzadeh et al., 2022)**.

Characterization of the biosynthesized SiO_2NPs

Double beam spectrum UV-Vis spectrophotometer V-760 (JASCO, UK) was used to monitor the production of SiO_2 NPs. The FT/IR-4100typeA was used to analyze the Fourier transform infrared spectroscopy (FT-IR) of SiO_2 NPs. An X-ray diffractometer (model LabX XRD-6000, Shimadzu, Japan) was also used to record the SiO_2 NPs X-ray diffraction patterns (XRD). Transmission electron microscopic (TEM) investigation was performed using TEM JEOL JEM-2100, Japan (200 kV). The surface charge and stability of SiO_2 NPs were measured using a Zeta potential analyzer (Malvern Zetasizer, Nano-ZS90, UK).

Antifungal activity agar well diffusion method

The fungicidal action of SiO_2 NPs alone or combined with the *M. chamomilla* L. extract against *A. niger* was demonstrated *in vitro* using an agar well diffusion test according to the guidelines of the Clinical and Laboratory Standards Institute **(CLSI, 2006)**. 100 µl from *M. chamomilla* L. extract, SiO_2 NPs, SiO_2 combined with the extract, Na_2SiO_3, and miconazole were prepared and added separately under aseptic conditions into 5mm wells in PDA plates before incubation at 30°C for 5 days. The inhibition zones were calculated in millimetres (mm).

The increase in fold area for SiO_2 NPs combined with chamomile extract was calculated according to the equation: $(B^2 - A^2)/A^2$; A is the inhibition zone of antifungal agents (SiO_2 NPs or chamomile extract) alone and B is SiO_2 NPs combined with chamomile extract, respectively **(El-Dein et al., 2021)**.

Radial mycelial growth inhibition

The inhibition of fungal radial growth was investigated according to **Quiroga et al. (2004)**. Briefly, a 5 mm disc of 7-day culture was cut and placed upside down in the centre of PDA plates amended with 200 µl of the tested antifungal agents (100 µg/ml) dissolved in 70% of ethanol; *M. chamomilla* L. extract, SiO_2 NPs, and SiO_2 NPs combined with the extract. Inoculated PDA plates without antifungal agents were used as a control. The plates were incubated at 30°C for 7 days. The average diameter of the growth was measured in mm and percent inhibition rates were calculated through the following formula: Inhibition rate (%) = (R – r) / R Where R represents the fungal radial growth on the control. In contrast, r represents the fungal radial growth on the treated plate.

Statistical analysis

The ANOVA test was performed to analyse the findings with SPSS software version 18. The significance level was set at 0.05. The trials were repeated three times. All results were provided as the mean and standard deviation (SD) **(O'Connor, 2000)**.

RESULTS AND DISCUSSION

Morphological identification of fungal isolates

Aspergillus spp. was found to be the most prevalent fungus in yellow corn samples (70.2%), followed by *Penicillium* (14.19%), *Alternaria* (10.3%), and *Fusarium* spp. (5.31%) (Figure 1). Similarly, **Abe et al. (2015)** reported that the majority of the isolated and detected fungal genera in maize were *Aspergillus*, *Fusarium*, *Penicillium*, *Alternaria*, *Cladosporium*, *Curvularia*, *Mucor*, and *Trichoderma*. Identification of fungal isolates based on the morphological characteristics of different fungal genera. *Aspergillus* section *Nigri* was likewise the most common among other *Aspergillus* species, with only one species identified, *A. niger*. Whereas *Penicillium*, *Alternaria*, and *Fusarium*, were recognized by genus rather than species. *A. niger* exhibited a black colony that propagated radially from the spot of inoculation, according to macroscopic examination. The colony gradually grew to fill the whole Petri dish, with underlying, white mycelium topped by a layer of densely packed. Black conidial heads and reverse pallid were also observed. *A. niger* colonies indicated that it is originally white, but soon becomes black with conidial production. Microscopic morphology revealed conidiophores borne from surface hyphae, with hyaline, heavy, smooth walls; vesicles spherical, containing densely packed metulae and phialides across the whole surface. The conidia were brown, spherical, with visibly roughened walls, radiating heads and borne in big.

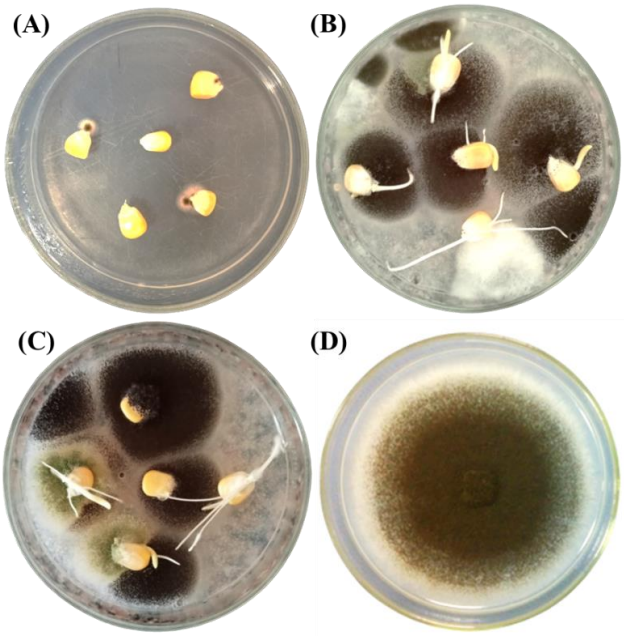

Figure 1 Colonies of *A. niger*, *A. flavus*, *Alternaria* spp., *Penicillium* spp., and *Fusarium* spp. isolated from yellow corn on PDA incubated at 30°C for two; (A) and five days; (B &C). Purification of *A. niger* at 30°C for seven days; (D).

Biosynthesis and characterization of SiO_2NPs

Ethnomedicinal extract of *M. chamomilla* L. was preferred to aqueous extraction and used in phytochemistry and pharmacological applications due to the successful extraction of the existence of major bioactive compounds **(Alhazmi et al., 2022)**. Moreover, *M. chamomilla* L. extract is rich in flavonoids, polyphenols, terpenoids, and sugars that act as bio-reducing agents during the biosynthesis of the NPs process **(Alshehri & Malik, 2020; Dogru et al., 2017)**. The formation of a white precipitate during the biosynthesis process was the first indication of the biosynthesis of SiO_2 NPs. UV-Vis spectrophotometer was used to analyse these solutions, which revealed strong three peaks at 266, 313, and 358 nm due to the surface plasmon resonance characteristic of the SiO_2 NPs in the reaction medium (Figure 2). **Park et al. (2010)** recorded two strong peaks of SiO_2 NPs at 215 and 280 nm while **Babu et al. (2018)** reported that SiO_2 NPs showed a broad adsorption peak at 450 nm.

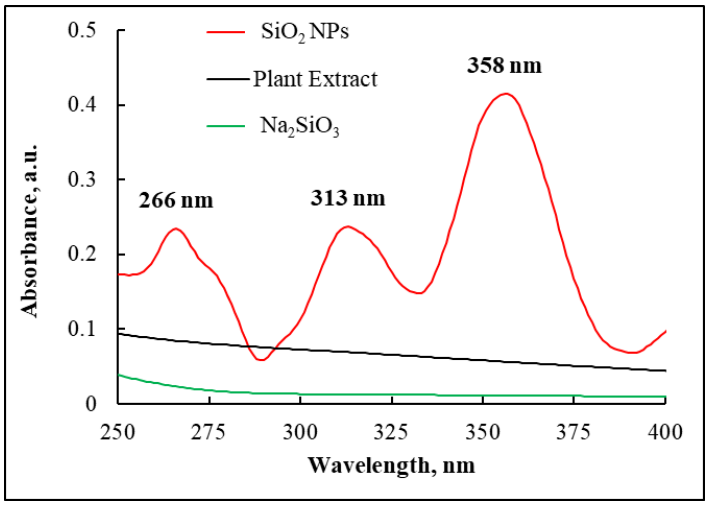

Figure 2 UV-visible spectroscopy of SiO_2 NPs compared to plant extract and Na_2SiO_3.

SiO_2 NPs FT-IR spectroscopic investigation revealed different peaks: 3402 cm^{-1} and 3306 cm^{-1} correspond to the -OH bond, 2925.48 cm^{-1}, which corresponds to the -C-CH_3- bond, 2857.02 cm^{-1} corresponds to the -C-CH_2- bond, 1635 cm^{-1} substituted cis-tri and vinyl absorption in addition to 1418 cm^{-1} and 1234.1 cm^{-1} corresponds to amines stretching vibrations. The C–O bond stretching appeared at 1034.2 cm^{-1}. While 1012.36 cm^{-1} and 530.13 cm^{-1} correspond to Si-O bending vibration, in which the oxygen atom moves perpendicular to the Si-O-Si plane (very strong), and 705.21 cm^{-1} corresponds to -C-CH_2- bond (Figure 3). These phytochemicals stabilize the NPs and prevent them from clumping together. **Goswami & Mathur (2022)** confirmed the existence of chemical composition and functional groups of the bio-generated SiO_2 NPs using the FT-IR spectrum. Aside from that, a band at 1645 cm^{-1} was found in the plant extract spectrum, which corresponded to silanol OH group adsorption. Furthermore, the FT-IR data revealed the presence of several proteins that act as capping and stabilizing agents **(Elazab et al., 2023)**.

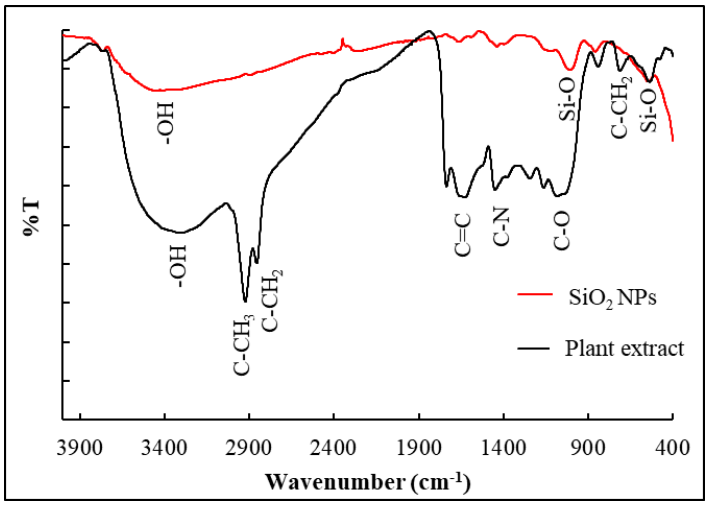

Figure 3 FT-IR spectra of plant extract and SiO_2 NPs.

Zeta potential examination of the produced SiO_2 NPs indicated a zeta potential value of -31.0 mV (Figure 4). The previous findings showed the good stability of biosynthesized SiO_2 NPs due to substantial repulsion forces between the particles caused by their high surface negative charge values, which inhibit aggregation and agglomeration.

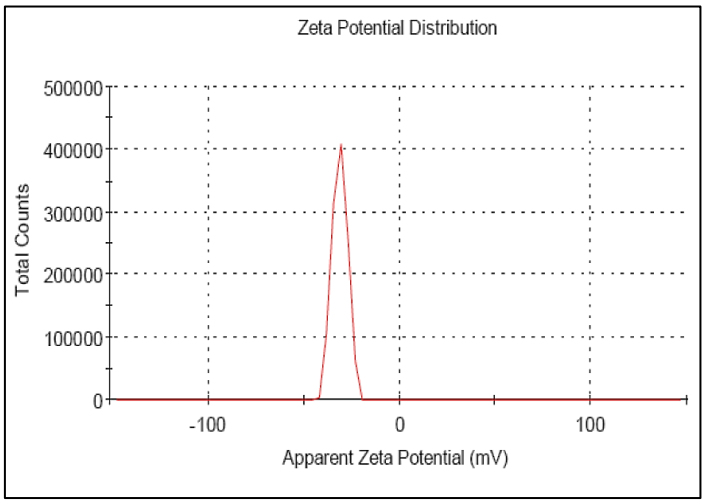

Figure 4 Zeta potential analysis of SiO_2 NPs.

The TEM micrograph revealed a good dispersion of the biosynthesized spherical-shaped SiO_2 NPs with an average size of 17-28 nm (Figure 5). **Al-Azawi et al. (2019)** attained spherical-shaped SiO_2 NPs with a 33.94 nm range utilising an aqueous extract of *Thuja orientalis* leaf. **Rahimzadeh et al. (2022)** used *Rhus coriaria* L. extract in the green synthesis of SiO_2 NPs and compared them with chemically synthesized SiO_2 NPs. In both cases, the results revealed that the SiO_2 NPs had a spherical form. The chemically synthesized SiO_2 NPs had an average size of approximately 30 nm, whereas the green synthesized SiO_2 NPs had an average size of around 55 nm, and the NPs were amorphous.

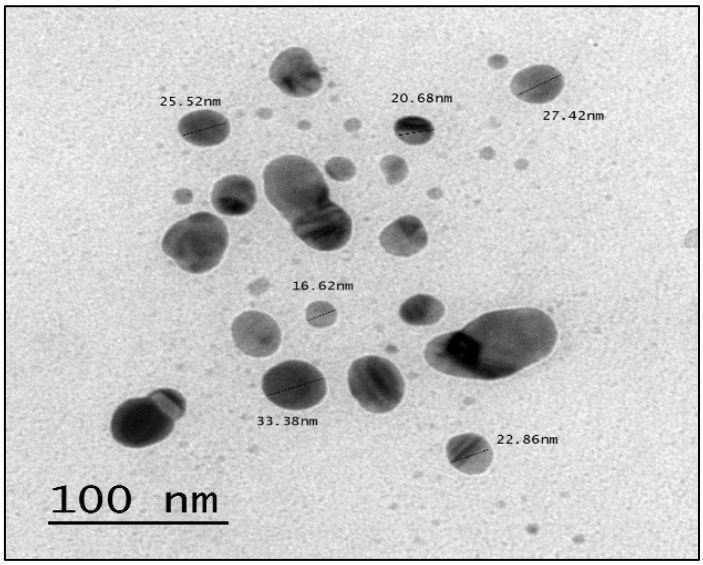

Figure 5 TEM of the biosynthesized SiO_2 NPs using *M. chamomilla* L. extract (bar scale = 100 nm).

Figure 6 depicts the XRD pattern of SiO_2 NPs; significant diffraction peaks of SiO_2 NPs were found at $2\theta = 32°$, 46°, 57°, 66°, and 76°. The recorded diffraction peaks were similar to **Goswami & Mathur (2022)** results that proved the crystallographic structure of SiO_2 NPs. Debye-Scherrer equation was used to determine the size of the SiO_2 NPs ($D = k\lambda/\beta cos\theta$; D; average crystalline particle size, k; Scherer's constant (0.9), λ; wavelength of x-ray (1.5406 Å), β; XRD peak full width at half maximum, and θ; diffraction angle). The mean size was found to be 22.7 nm, which resembled the TEM data.

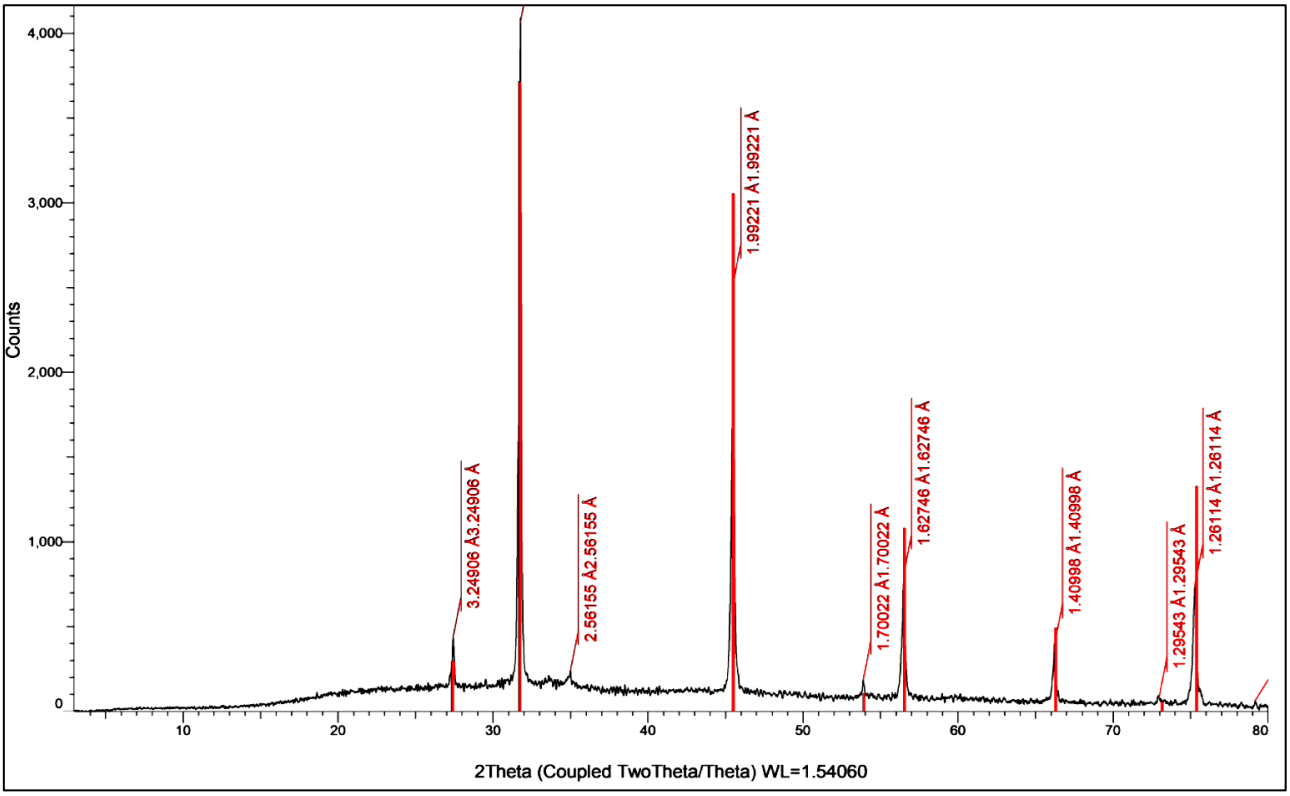

Figure 6 XRD spectrum of the biosynthesized SiO_2 NPs using *M. chamomilla* L. extract.

Antifungal efficiency of *M. chamomilla* L. extract alone or combined with SiO_2 NPs

The biosynthesized *M. chamomilla* L. extract, SiO_2 NPs and SiO_2 NPs combined with *M. chamomilla* L. extract showed higher antifungal activity against *A. niger* than Miconazole as shown in Figures 7 &8. The biosynthesized SiO_2 NPs had substantial synergistic effects when combined with chamomile extract, in addition to its antifungal properties, which increased its fold area by 1.16. These findings confirmed the potential for employing biosynthesized SiO_2 NPs as promising alone or mixed antifungal agents in various medicinal and industrial applications. According to **EL-Hefny et al. (2019)**, essential and recovery oils derived from *M. chamomilla* fresh flowers have been found to have antifungal activity against fungi related to biodeterioration of cultural assets (*A. niger, A. terreus, A. flavus, and F. culmorum)*. The study also documented that the antifungal action of essential oils of *M. chamomilla* was dose-dependent, with the best results against *A. niger*.

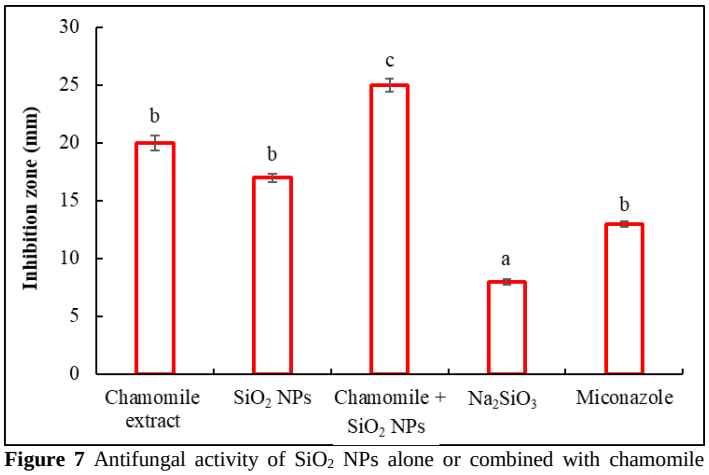

Figure 7 Antifungal activity of SiO_2 NPs alone or combined with chamomile extract against *A. niger* in comparison with miconazole as a standard antifungal agent.

The dried flowers of chamomile were hydrodistilated and analyzed for essential oil compounds as studied by **Chauhan et al. (2022)**. It was confirmed the presence of bisabolol oxides A and B, azulene-7-ethyl-1,4-dimethyl, trans-β-farnesene, limonene, isobornyl isobutyrate, and bisabolone oxide as essential oil compounds. In comparison, **Amiri & Sharafzadeh (2014)** identified chamazulene, α-bisabolol oxide A, α-bisabolone oxide A, en-yn-dicycloether, α-bisabolol oxide B, n-octanal, germacrene D α-terpineol, and 1,8-cineole as phyto-bioactive compounds. On the other hand, the antimicrobial activity of SiO_2 NPs synthesized using *A. niger* inhibited the *A. Sydowii* with inhibition zone 27 mm as reported by **Alhazmi (2023)**. It is thought that chamomile-extract essential oils act on the cell cytoplasmic membrane and cause stress in microorganisms. At subinhibitory concentrations, essential oils could cause oxidative stress, metabolic interference, cell wall disruption, and ultimately cell death **(Das et al., 2019)**.

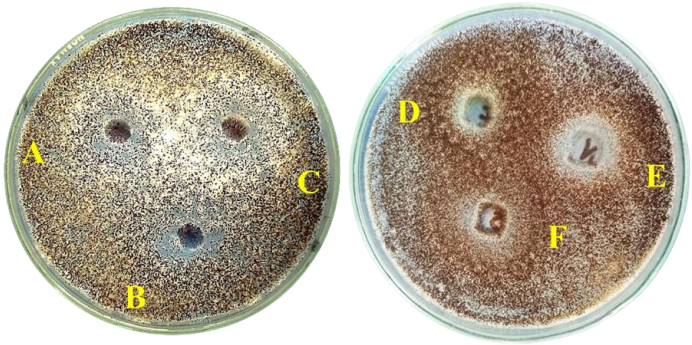

Figure 8 Antifungal activity of chamomile extract alone; (A), SiO_2 NPs; (B), chamomile extract + SiO_2 NPs; (C), Na_2SiO_3 ;(D) and miconazole; (E) against *A. niger*. (F) Ethanol (70%) as a negative control.

Figure 9 displayed the *A. niger* mycelium radial growth and inhibition rate percent during treatment using tested antifungal agents, including chamomile extract alone and chamomile extract combined with SiO_2 NPs. SiO_2 NPs combined with chamomile extract showed higher antifungal action against *A. niger* with an inhibition rate of 34% than chamomile extract alone which inhibited the fungal growth by 16%. **Goswami & Mathur (2022)** reported that *A. niger* and *F. oxysporum* were suppressed by 1000 µg/l of SiO_2 NPs derived from agricultural waste (Sugarcane bagasse and corn cob) with mycelia growth inhibition 58.92% and 73.42%, respectively. In comparison to conventional emulsions and free oils, SiO_2 NPs act as a stabilizer, preventing the simple escape of essential oils from the emulsion system **(Mariyate & Bera 2021)**. **Masoud et al. (2022)** said that the combination of bio-silica NPs and *Cassia nodosa* extract increased the inhibition action of some fungal strains like *F. oxysporum* and *R. solani*.

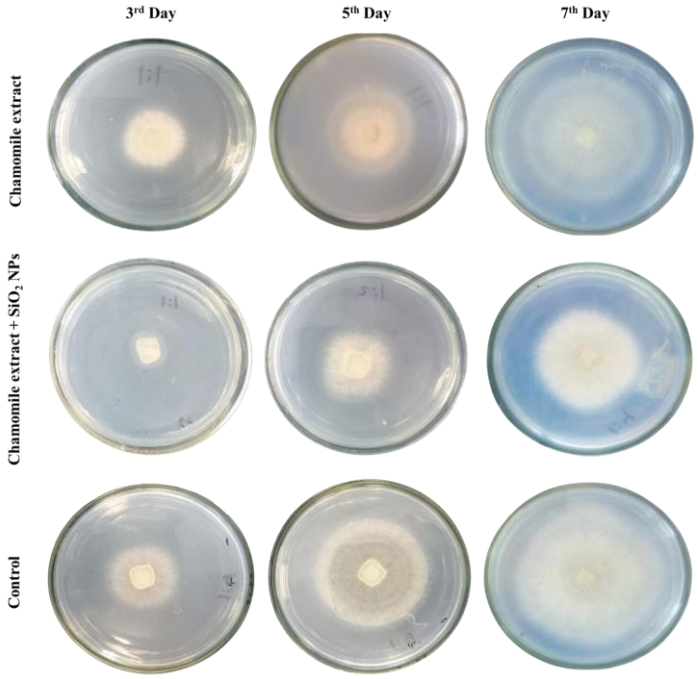

Figure 9 Radial mycelial growth of *A. niger* in the presence or absence of chamomile extract alone or chamomile extract + SiO_2 NPs at a concentration of 100µg/ml.

CONCLUSION

A. niger was isolated, identified and selected for studying the antifungal activity. SiO_2 NPs were biosynthesized using the extract of *M. chamomilla* L. as a bio-reducing agent. The provided biosynthesis approach was rapid, simple, cheap, and eco-friendly with high stability. SiO_2 NPs production was confirmed and characterized using UV-Vis, FT-IR and XRD spectral analyses and TEM and Zeta potential analysis. To the best of our knowledge, this is the first report on the antimicrobial study of a combination of *M. chamomilla* L. extract and SiO_2 NPs. The biosynthesized NPs combined with chamomile extract had higher antifungal activity against *A. niger* than individual SiO_2 NPs or chamomile extract.

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