Plant Protection (Scientific Journal of Agriculture)

49(2), Summer, 2026

10.22055/ppr.2026.51595.1878

The control efficiency of *Urtica dioica* extract loaded in silica nanoparticles against wheat take-all disease caused by *Gaeumannomyces graminis* var. *tritici*

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Received: 6 January 2026 Revised: 3 February 2026 Accepted: 23 February 2026

Nettle extract has been recorded as a plant growth stimulator as well as pest and disease inhibitor. The extract of leaves of *Urtica dioica* (UDE) was extracted using ethanol, with chemical compounds identified via gas chromatography-mass spectroscopy (GC-MS) analysis. The UDE was then loaded into mesoporous silica nanoparticles (MSNPs) as carrier and was tested against *Gaeumannomyces graminis* var. *tritici* (*Ggt*), both *in vitro* and *in vivo*. The effect of pure extract and the extract loaded in MSNPs (UDE-MSNPs) was assessed on the growth parameters of wheat plants and seed germination. Fifteen different compounds of UDE, constituting approximately 94 % of the extract, were identified by GC-MS. The complete inhibition of *Ggt* growth *in vitro* occurred at 1.2 mg/mL of the pure extract, which diminished significantly to 0.6 mg/mL after loading into MSNPs. Seed treatment with the pure UDE (1.2 mg/mL) and UDE-MSNPs (0.6 mg/mL) led to 100% seed germination and in the presence of UDE-MSNPS, the seeds germinated faster. The seed treatment with pure UDE and UDE-MSNPs at MFC reduced the severity of wheat take-all disease up to 51.54 % and 63.65 %, respectively compared with mancozeb fungicide (69.16 %). Meanwhile, biocontrol efficacy on wheat plants in soil method by pure UDE and UDE-MSNPs was 78.54 % and 80.25%, respectively. Further, in inoculated and non-inoculated plants, the influence of the pure and extract-loaded in MSNPs in soil on the fresh and dry weight of the roots was prominent and more noticeable in the case of loaded extract. MSNPs also extended the shelf life of UDE from 9 to 29 days, causing gradual release of the extract into the soil. Thus, MSNPs improved shelf life of extract, controlled delivery, enhanced efficacy, and lowered disease severity of wheat take-all disease at a lower concentration (0.6 mg/mL) in both seed and soil treatments compared with pure forms (1.2 mg/mL).

Keywords: Natural plant products, Nettle extract, GC-MS, MFC

Associate editor: M. Ziaee (Prof.)

Citation: Sattary, M., Amini, J., & Hallaj, R. (2026). The control efficiency of *Urtica dioica* extract loaded in silica nanoparticles against wheat take-all disease caused by *Gaeumannomyces graminis* var. *tritici*. *Plant Protection (Scientific Journal of Agriculture), 49*(2), 93-115. https://doi.org/ 10.22055/ppr.2026.51595.1878

گیاه‌پزشکی (مجله علمی کشاورزی)
جلد ۴۹، شماره ۲، تابستان ۱۴۰۵

10.22055/ppr.2026.51595.1878

تاثیر بازدارندگی عصاره خالص و نانوکپسوله شده گیاه گزنه (*Urtica dioica*) روی بیماری پاخوره گندم ناشی از قارچ *Gaeumannomyces graminis* var. *tritici*

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تاریخ دریافت: ۱۴۰۴/۱۰/۱۶ تاریخ بازنگری: ۱۴۰۴/۱۱/۱۴ تاریخ پذیرش: ۱۴۰۴/۱۲/۰۴

چکیده

عصاره گیاه گزنه سبب تحریک رشد گیاهان می‌شود و از گیاهان در مقابل آفات و بیماری‌ها محافظت می کند. عصاره اتانلی از برگ گزنه جداسازی و ترکیبات آن بوسیله کروماتوگرافی گازی- طیف سنجی جرمی (GC-MS) شناسایی گردید. عصاره گیاه گزنه در مزوپورهای سیلیکا بارگیری گردید و تاثیرات آن در شرایط آزمایشگاه و گلخانه روی قارچ *Gaeumannomyces graminis* var. *tritici* (*Ggt*) عامل بیماری پاخوره گندم مورد بررسی قرار گرفت. همچنین ترکیب خالص و نانوکپسوله شده گیاه گزنه روی فاکتورهای رشدی و جوانه زنی بذر گندم بررسی شد. پانزده ترکیب مختلف از عصاره گزنه با روش GC-MS جداسازی گردید. در شرایط آزمایشگاه دو غلظت ۱/۲ میلی‌گرم در میلی‌لیتر و ۰/٦ میلی‌گرم در میلی‌لیتر عصاره خالص و نانوکپسوله شده گزنه به ترتیب موجب جلوگیری از رشد کامل میسلیوم قارچ پاخوره گندم شدند. تیمار بذر گندم با عصاره خالص و نانوکپسوله شده گزنه بترتیب با ۱/۲ و ۰/٦ میلی‌گرم در میلی‌لیتر سبب صد درصد جوانه زنی بذر گندم شدند. همچنین تیمار بذر گندم با عصاره خالص و نانوکپسوله شده گزنه در کمترین غلظت قارچکشی بترتیب سبب کاهش ٥١/٥٤ و ٦٣/٦٥ درصد شدت بیماری پاخوره گندم در مقایسه با تیمار قارچکش مانکوزب (٦٩/١٦ درصد) شدند. در صورتیکه، تیمار خاک با عصاره خالص و نانوکپسوله شده گزنه سبب کاهش شدت بیماری پاخوره گندم به‌ترتیب به میزان ٧٨/٥٤ و ٨٠/٢٥ درصد گردید. بعلاوه، تاثیرات عصاره گزنه نانوکپسوله شده در خاک روی فاکتورهای رشدی گیاه گندم مثل وزن تر و خشک ریشه بیشتر از عصاره خالص گزنه بود. نتایج نشان داد که پایداری ترکیب نانوکپسوله شده عصاره گزنه در مقایسه با عصاره خالص در خاک از ۹ روز به ۲۹ روز افزایش یافت. بنابر این، نانوکپسوله عصاره گیاه گزنه سبب بهبود عملکرد، پایداری عصاره و کنترل رهایش آن در محیط گردید و شدت بیماری پاخوره گندم را در غلظت پایین (٠/٦ میلی گرم در میلی لیتر) در هر دو روش تیمار خاک و بذر در مقایسه با عصاره خالص (۱/۲ میلی گرم در میلی لیتر) بیشتر کاهش داد.

کلید واژه‌ها: تولیدات طبیعی گیاه، گزنه، GC-MS، MFC

دبیر تخصصی: دکتر معصومه ضیائی

Introduction

Take-all disease, caused by *Gauemannomyces graminis* var. *tritici* Walker (*Ggt*), is one of the most destructive root diseases of wheat worldwide (Zhang et al., 2020). As with other soil-borne diseases, take-all is difficult to control because, traditional methods such as crop rotation, breeding for resistant plant cultivars as well as application of fungicides, have been insufficient to management of take-all disease (Jolideh et al., 2012; McMillan et al., 2018; Ramanauskienė et al., 2018). One environmentally-friendly method to controlling the fungal pathogens can be the application of plant secondary metabolites such as plant extracts as well as essential oils (EOs) (Hosseni et al., 2020; Weisany et al., 2022; Javanmardi et al., 2023). Plants produce a wide range of secondary metabolites, including phenols and polyphenols, terpenoids, alkaloids, saponins, steroids, lectines, fatty acids and polypeptides, which can be utilized as botanical fungicides for management of plant pathogens (Hoseini & Amini, 2019; Ginovyan et al., 2020). Plant extracts have the potential to replace synthetic fungicides as they have no side effects, are easily degradable, and do not cause environmental pollution (Panda and Rath, 2012). Furthermore, they have a lower risk of pathogen resistance than chemical fungicides since they are composed of a combination of various biologically active compounds which probably have multiple targets in the pathogen cell many of which act with more than one mechanism (Gorlenko et al., 2020). Studies showed that concentrations of 7.5%, 10%, and 15% tea seed extract resulted in 100% mortality of second-stage larvae and inhibited the hatching of *Meloidogyne incognita* nematode eggs (Rahemi et al., 2025). Extract and nano extract of *Callistemon citrinus* and *Oliveral decumbens* reduced citrus green mold disease caused by *Penicillium digitatum*, so that the nano-extracts of both plants were more effective against *P. digitatum* than their common extract counterparts (Helalipour et al., 2025). Shirazi Thyme extracts reduced mycelium growth of *Fusarium oxysporum* f. sp. *lycopersici in vitro* and decreased disease severity of tomato wilt under greenhouse conditions (Ghazalbashi et al., 2014). Also, the garlic and thyme extracts decreased fungal mycelium growth of *Penicillium digitatum* causal agent of orange green mold *in vitro* and enzyme activity of peroxidase and polyphenol oxidase were increased after 9 days (Gholamnezhad et al., 2019).

Stinging nettle (*Urtica dioica* L.) is a perennial medicinal plant belonging to the *Urticaceae* family (Shabani et al., 2023). The phenolic compounds in nettle extract include ferulic acid, sinapic acid, caffeic acid, myrstin and phoctin, which have been recorded to offer antioxidant, antimicrobial, antifungal, and antibacterial properties (Chahardehi et al., 2009; Khan et al., 2023; Shabani et al., 2023).

Nano-based formulations can provide targeted delivery, controlled release, enhanced solubility, physiochemical stability as well as effective compounds. Mesoporous silica nanoparticles (MSNPs) are attractive bio-fungicides delivery vehicles thanks to several excellent properties which make them a promising candidate for usage in agriculture including: large surface area, ease of surface modifications, adjustable pore size and shape, high surface reactivity, and allowing the loading of small molecules as well as large proteins (Jain & Sharma, 2017; Weisany et al., 2019). Mesoporous silica nanoparticles (MSNPs) protect plants and boost crop yields through controlled delivery of biocides that are effective on pathogens and safe for the environment, thereby supporting sustainable agriculture. In addition, silica is naturally present in the soil and plays an important physiological role in

the plant, while lowering susceptibility of plants to fungal pathogens (Rai et al., 2015).

Nevertheless, to the best of our knowledge, there is no previous report of the antifungal study of nettle extract on wheat take-all disease. Thus, in the present study, the effect of *U. dioica* extract (UDE) in pure form and loaded in MSNPs (UDE-MSNPs) was ascertained for antifungal activity against *Ggt*. The objectives of the present study were to: i) investigate of inhibitory effects of different concentrations of pure UDE and UDE-MSNPs on *Ggt* mycelial growth *in vitro*, ii) determine the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of pure UDE as well as UDE-MSNPs, iii) along with their effects on controlling take-all disease as seed and soil treatment under controlled conditions, and iv) appraise the effectiveness of pure UDE and UDE-MSNPs on growth parameters of the wheat plants.

Materials and Methods

Fungal pathogen

The aggressive fungal pathogen (*Ggt*, GenBank accession number: MH488732) was obtained from the department of Plant Pathology, University of Kurdistan. The fungus was re-cultured on potato dextrose agar (PDA) prior to testing.

Plant material and extraction

The aerial parts of the *U. dioica* were collected in Mahmoudabad city located in Mazandaran province, north of Iran. The plant samples were washed with distilled water, dried in the shade at room temperature, and were milled to a fine powder using a grinder. The extract was obtained through the maceration method with 70% ethanol solvent as described follows: the powdered herbs and solvent were mixed (1gr/10 mL) in a dark bottle at a ratio of 1:10 and were shaken (Pole ideal Tajhz co, model: PIT10LO-Z10-230 VAC) at 100 rpm for 48 h at room temperature. Next, the obtained extract was filtered through a Whatman's No. 1 filter paper with the solvent evaporated to dryness using a rotary evaporator (Heidolph, Hei-VAP Value, Germany) at 40 °C. The concentrated extract was stored at 4°C until use (Florez et al., 2022).

Gas chromatographic–mass spectrometry (GC–MS)

For identifying the UDE compounds, an Agilent 7890A gas chromatography (GC) coupled with Agilent 5975C mass spectrometry (MS) (Agilent Technologies, USA) was employed (In Nanotechnology Research Center, University of Kurdistan) using an HP-5 MS capillary column (30 m × 0.25 mm, 0.25 μm film thickness). The GC-MS conditions and temperatures have been reported elsewhere (Sattary et al., 2020).

Loading of UDE onto MSNPs

For synthesis of the MSNPs, 2 g of CTAB as the structure-directing agent was dissolved in 960 mL of deionized water to which 7 mL of 2 M NaOH was added with the temperature of the solution adjusted at 80 °C. Thereafter, 5 mL of TEOS as silica source was added dropwise under intense stirring with the solution stirred for 2 h. At last, the resulting white precipitate was separated through centrifugation (500 0 rpm, 10 min) and was well washed with deionized and ethanol. Finally, it was dried in vacuum at 75°C for 18 h to obtain MSNPs and calcined at 550 °C for 5 h in the air (Melendez-Rodriguez et al., 2019).

Characterization of loaded MSNPs

Morphology and particle size measurement

The morphology and particle size of loaded MSNPs were imaged through scanning electron Microscopy (SEM) using TESCAN MIRA3, Czech Republic at a voltage of 30 kV and transmission electron Microscopy (TEM) using Philips Microscope, EM 208, Tokyo, Japan at 100 kV.

Loading capacity

A UV-Vis-Spectrophotometer (Bruker Model Vector 22) was utilized to inspect the UDE content in MSNPs. Initially, a calibration curve was created based on the absorption at maximum wavelength using different amounts of pure UDE in 2 mL acetonitrile. Supernatant adsorption was then read at 196 nm using a UV-Vis-Spectrophotometer. The amount of UDE loaded in the MSNPs was extrapolated to the calibration curve of pure UDE through subtracting the residual amount from what was initially added.

Release of UDE of MSNPs

In order to examine the kinetics of the UDE release from MSNPs in water and soil, the UDE-MSNPs were suspended in 5 mL of sterile deionized water or were mixed with 100 gr soil at 25°C for 37 days. Next, 5 mL of the release water medium was removed for analysis at four-day intervals, and replaced with 5 ml of fresh deionized water. Considering soil, irrigation was performed at same given times whereby the water coming from the bottom drain holes was filtered, centrifuged (10,000 rpm for 10 min), with the UDE concentration measured by means of UV–Vis based on the UDE absorbance at 196 nm. Pure extract according to the amount of loaded in MSNPs, were used as a control (Sattary et al., 2020). Release of *Urtica dioica* extract from mesoporous silica nanoparticles in water and soil was measured on days 1, 5, 9, 13, 17, 21, 25, and 29. The experiment was performed in eight treatments with four replicates (four samples per days). The release was quantified from the following equation (Wanyika, 2013):

(i) Release (%) = (Released oil/Total oil) × 100

***In vitro* antifungal activity**

In order to determine the antifungal activity of the UDE and UDE-MSNPs against *Ggt*, a stock solution of the UDE (100 mg/mL) was prepared through dissolving the dried extract in sterile distilled water. Thereafter, different concentrations (0.12, 0.39, 0.66, 0.93, and 1.2 mg/mL) based on pre-tests were provided though diluting the stock solution in 100 mL sterile melted potato dextrose agar (PDA) at 40 °C (Robertson et al., 2007). The mixture of plant extract and PDA (~20 mL) was poured into sterilized glass petri dishes (90 mm diameter) and shaken for 5 min. To UDE-MSNPs, 20 mL of PDA was poured into sterilized petri plates whereby the required mass of loaded extract was supplemented to prepare the following concentrations (0.12, 0.24, 0.36, 0.48, and 0.6 mg/mL). Extract-free PDA was employed as a control. After solidifying of PDA, 5 mm disks from the edge of the 7-day-old culture of *Ggt* was placed onto the center of the PDA. Subsequently, inoculated plates were sealed with parafilm and incubated in the darkness at 27±2 °C for 10 days. When the colonies of the control completely covered the plate, the diameter of fungal growth was measured in the sample and control plates (Jing et al., 2017; Hoseini & Amini, 2019). The formula for calculating the percentage inhibition of radial growth (PIRG) was as follows:

(ii) $PIRG = [(R1-R2) /R1] \times 100$

Where R1 represents the radial growth of pathogen in control plate and R2 denotes the radial growth of pathogen in the extract treated plate. Experiments were designed as completely randomized design with five replicates.

Determination of minimum inhibition concentration (MIC) and minimum fungicidal concentrations (MFC)

The MIC was defined as the lowest concentration of the extract that would completely inhibit the growth of *Ggt* as compared to the control. In order to decide whether the extract is fungistatic or fungicidal in minimum inhibition concentration (MIC), the inhibited fungal disks were re-cultured onto fresh PDA

medium lacking extract and incubated for 10 days at 27±2 °C. The minimum fungicidal concentrations (MFC) were considered as the lowest extract concentration after which no visible growth occurs after re-inoculation (Jing et al., 2017; Hoseini & Amini, 2019).

Effect of the pure and loaded UDE on mycelium morphology

For observing the effect of the pure UDE and UDE-MSNPs on the hyphal morphology, a small piece of fungal mycelium treated with an MFC of the pure and loaded extract or without any treatment (control) was fixed on the glass slide. After seven days, microscopic examination was performed using optical Microscope Olympus BX-51 and analyzed via Olympus cell Sens Entry software.

***In vivo* experiments**

Seed germination test

In order to find the optimal concentration of the UDE for seed treatment, seed germination test was performed. One hundred surface sterilized wheat seeds (*Triticum aestivum* c.v Pishgam) were soaked in 5 ml of different pure UDE concentrations (1.2, 2.4, and 3.6 mg/mL) as well as UDE-MSNPs at 0.6 (the concentrations were selected based on MFC concentration). Seeds soaked in sterile water were employed as a control water while seeds soaking in empty MSNPs in an equal quantity of UDE-MSNPs were applied as a MSNPs control. Next, all pots were incubated in the growth chamber under 12 th alternating cycles of light and darkness at 25 ± 2 °C. Two germination factors, seed germination percentage and speed of germination, were calculated using the following formulas after nine days (Gairola et al., 2011):

i)

$$Germination\ (\%) = \frac{Number\ of\ germination\ seeds}{Total\ unmber\ of\ seeds} \times 100$$

ii) Speed of germination= n1/d1+ n2/d2+ n3/d3+ ---------

Where, n = number of germinated seeds, d= number of days.

Pot experiments

To research the antifungal influence of the pure UDE and UDE-MSNPs on wheat take-all disease, evaluation was measured in pot experiments as a seed and soil treatment under controlled conditions. For preparing the inoculum, autoclaved moistened wheat seeds were inoculated by pieces of *Ggt* agar culture and incubated in darkness at 25 ± 2 °C for four weeks. The inoculated seeds were blended with steam-sterilized mixture of clay, sand, and silt (1:1:1, w: w: w) and at the rate of 1% (w: w). The pots with a 300 mL volume were employed in these tests and three wheat seeds (*Triticum aestivum* c.v Pishgam) were planted in each pot (five pots were used in each treatment). After 35 days, the disease index was appraised based pn the scaling system from 0-5 was used according to Gholami et al. (2019).

Seed treatment

To ascertain the antifungal activity of the pure and loaded extract against *Ggt*, surface disinfected wheat seeds were dipped in five mL of the pure and loaded extract at 1.2 and 0.6 mg/mL, respectively for 12 h. Next, inoculated seeds were planted in pots containing sterile soil (five pots were used in each treatment and three seeds were planted per pots). The treatments were as follows:

a) Pure UDE + *Ggt* (to ascertain the effect of the extract on *Ggt* development)
b) Pure UDE (to appraise the impact of the extract on growth parameters of wheat plants)
c) UDE- MSNPs + *Ggt* (to assess the influence of the loaded extract on *Ggt* development)
d) UDE- MSNPs (to evaluate the effect of the loaded extract on growth parameters of wheat plants)
e) MSNPs without UDE + *Ggt* (to appraise the effect of the empty MSNPs on *Ggt* development)

f) Empty MSNPs (to ascertain the effect of the empty MSNPs on growth parameters of wheat plants)
g) Sterile distilled water + *Ggt* (inoculated control)
h) Sterile distilled water (non-inoculated control)
i) The fungicide of Mancozeb (80% a.i. WP; manganese/zinc ethylene bis-dithiocarbamate; China product; Cas number: 8018-01-7; 2 gr mancozeb/ kg wheat seed) + *Ggt* as a fungicide control.

These treatments were also applied for the soil treatment.

Soil treatment

The selected concentration for seed application with the same treatments was also employed for the soil amendments. The concentration of fungicides applied in the soil treatment was 0.013 mg/mL (Sattary et al., 2020). The treatments were added to the soil in pots at the rate of 10 mL per 300 mL soil according to Sharma et al. (2018). Three coated seeds were sown per each pot with five pots used for each treatment. The experiments were replicated twice. The plants were grown under controlled conditions (in the department of Plant protection, Agriculture faculty, University of Kurdistan) at 25 ± 2 °C, 60–70% RH, 12 h light, and 12 h darkness and watered every 4 days. After 35 days of planting, the plants were removed from the soil whereby the growth parameters (shoot and root length, shoot and root fresh weight, shoot and root dried weight) were measured. For evaluating the disease severity, the scaling system from 0-5 was employed according to Gholami et al. (2019), where 0 = no disease, 1 = 1-25 % of root system affected, 2 = 25- 100 % of root system affected, 3= 100 % of root system affected with the black tissues extending into the stem base, 4 = black tissues developed on the stem and some coalescing, and 5 = plant extremely stunted or dead. The disease reduction percentage was then identified according to the following formula (Gholami et al., 2019):

$$\text{Disease reduction \%} = \frac{Disease\ index\ of\ control - disease\ index\ of\ treated}{disease\ index\ of\ control} \times 100$$

Nitrogen content measurement

In order to explore the effect of pure and loaded extract on nitrogen absorption by plant wheat and the nitrogen content of the aerial parts of the inoculated plants, the standard method for determining total N in plants was adopted in three steps according to Ferreira et al. (2015) with some modifications. The procedure involved digesting the dried plant tissue (1g) in concentrated sulfuric acid (20 mL) in the presence of two catalysts, $Na2SO_4$ and $CuSO_4$, distilling with NaOH (5 %, 75 mL) plus boric acid (2 %, 50 mL) and titrating with HCL (1 % N). Eventually, the percentage of nitrogen in the samples was calculated by the following equation:

v) N % = [14 × (A- B) × N × 100] / [1000 × weight of sample (g)]

Where A= Vol. of HCL (mL) used in the sample titration
B= Vol. of HCL (mL) used in the blank titration
N= Normality of the standard acid (eq/L)
14= Atomic weight of nitrogen

Statistical analyses

Statistics on a completely randomized design (each treatment with five replicates) were undertaken using variance analysis (ANOVA) in SAS software (version 9.1; SAS Institute Inc., Cary, NC, USA). To detect differences of mean data, Duncan's Multiple Range Test (DMRT) ($p < 0.05$) was employed with all data presented as mean ± standard error (SE).

Results and discussion

Identification of phytochemical compounds by GC–MS analysis

The GC-MS analysis of ethanolic extract of *U. dioica* identified presence of 15 phytochemical compounds from different chemical classes (Supplementary data, see Appendix 1). Phen-1,4-diol, 2,3-dimethyl-5-trifluoromethyl (46.29 %) and Benzaldehyde (0.48%) were the major and minor ingredients of the UDE, respectively. Benzaldehyde is an aldehyde found in the leaves of *U. dioica* (Alizadehdakhel & Golzary, 2020) which has antimicrobial, insecticidal, and antioxidant activities (Hameed et al., 2016; Ullah et al., 2015). Phen-1,4-diol, 2,3-dimethyl-5-trifluoromethyl is also a biochemical compound in plant extracts possessing antimicrobial, antioxidant, antithrombotic and antituberculosis activities (Hameed et al., 2015; Al-Marozoqi et al., 2016). Further antimicrobial compounds which were identified here included: Acetic acid (2.3 %), 2-Allyl-6-methoxyphenol (0.96 %), Eugenol (2.03 %), Diethyl Phthalate (11.94 %), Phthalic acid (10.28 %), Benzofuran-2-one, 4-amino-2,3-dihydro (5.56 %), Oxime, methoxy-phenyl (1.21%), Phenol, 2-methoxy-5-(1-propenyl)- (E) (2.74 %) and phytol (2.07%) (Al-tameme et al., 2015; Shareef et al., 2016) (Supplementary data, see Appendix 1).

Scanning electron microscopy (SEM) and Transmission electron microscopy (TEM)

Fig. 1 displays the SEM and TEM images of the UDE-MSNPs employed through this assay. The morphology and dimension of MSNPs were examined by SEM and TEM. Spherical particles with approximate diameter 70 nm and hexagonal straight channels can be observed in Fig. 1.

The UDE loading and release analysis

The UDE encapsulation efficiency was calculated as 64% using a standard curve y= 0.0478x+ 0.0687, R^2=0.9971 at 196 nm. As depicted in Fig. 2, the UDE release into water was far faster than that of soil. More than 98% of the extract was released into water within 21 days with six water replacements and in the soil for 29 days along eight soil irrigations. The extract release in water was 41.75% during the first replacement and 39.95% in soil along the first stage of irrigation. This release profile has been typically observed in other loaded MSNPs where the initial burst release has been assigned to the surface desorption of the cargo in external pores while subsequent slow release is owing to internal absorption into deep pores (Wu et al., 2019). The stability of pure extract in soil was nine days whereby 97.11% was released (data not shown). Thus, MSNPs improved the shelf life of extract, controlled delivery, and boosted efficacy which is consistent with previous studies (Worrall et al., 2018).

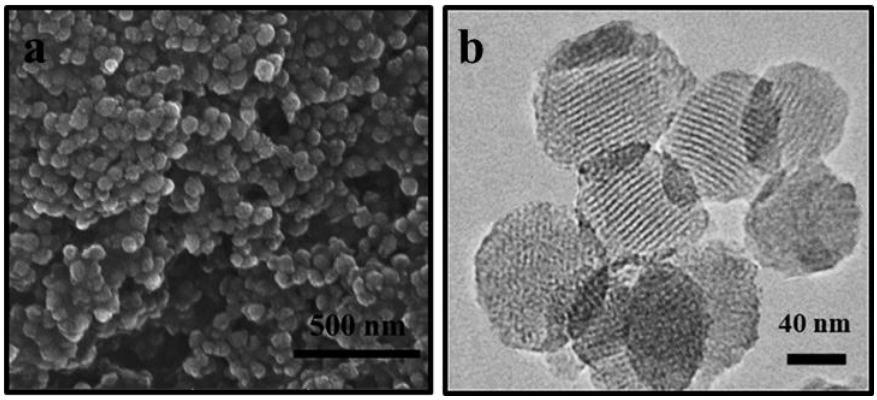


Figure 1. Scanning electron microscopy (SEM) image (a) and Transmission electron microscopy (TEM) image (b) of mesoporous silica nanoparticles loaded by *Urtica dioica* extract.

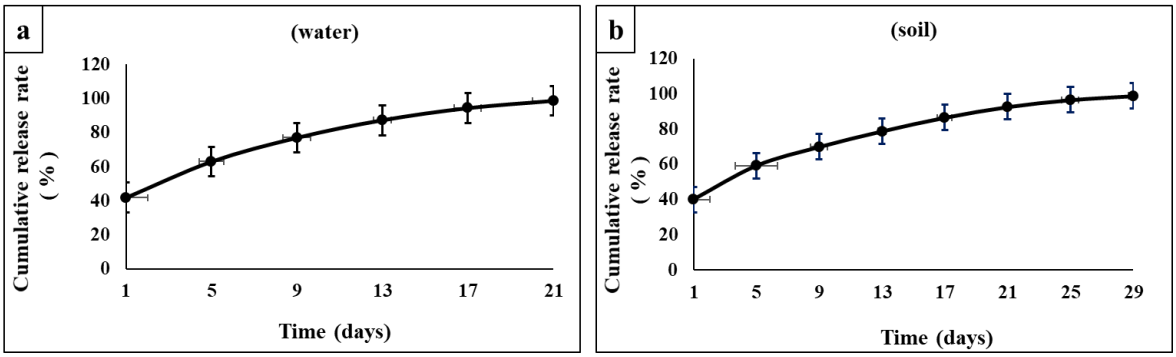


Figure 2. Release profile of *Urtica dioica* extract from mesoporous silica nanoparticles (a, in water and b in soil). Data are means of five replications± standard errors.

***In vitro* test**

Antifungal assay, MIC, and MFC

The antifungal activity was observed to be associated with the concentration, and significantly increased ($p < 0.01$) with augmentation of UDE contents (Table 1). Fungal growth was completely inhibited at the concentration of 1.2 mg/mL (MIC) of UDE whereby a fungicidal concentration was also found (MFC), since no growth was seen in the new PDA medium after *Ggt* re-culturing. The UDE- MSNPs remarkably lowered the fungistatic and fungicidal concentrations (MIC: 0.47 and MFC: 0.58 mg/mL), showing almost twice more antifungal activity than UDE. The UDE-MSNPs mitigated the fungal growth of *Ggt*, completely at the concentration of 0.48 mg/ml. The results of this test highlight the strong antifungal effect of the pure UDE and the role of MSNPs in boosting its antifungal activity (Table 1).

The literature has proven the antifungal activity of ethanolic extract of *U. dioica* (Mahmoudi et al., 2014). According to studies, encapsulation of essential oils and plant extracts boosts their solubility, chemical stability, and bioavailability, while also protecting them from oxidation (Sattary et al., 2020). The gradual release of antifungal compounds in the environment ameliorates shelf life and protection. Likewise, the high contact surface and small size of nanoparticles allow them to penetrate better into fungal cells, eventually leading to loss of membrane integrity, leakage of cellular contents, and fungal cell death. Thus, the loaded plant compounds demonstrate superior antifungal performance compared with pure forms, with lower concentrations being needed to inhibit the growth of the fungus (Janatova et al., 2015; Zhaveh et al., 2015).

Effect of the extract on mycelium morphology

The optical microscopic images of the fungal hyphae exposed to the MFC of the UDE and UDE- MSNPs are exhibited in Fig. 3. Light microscopy, in the control sample (not treated with UDE) indicated that the hyphae were thick, uniform, smooth-surfaced, and did not present any abnormality (Fig. 3a). The treatment with the pure UDE led to diminished hyphal diameter and cytoplasmic vacuolization (Fig. 3b). In the presence of loaded extract, severe hyphal deformities such as abnormal branching, hyphal lysis, cell wall disruption, cytoplasmic coagulation, and irregular cell masses were observed (Fig. 3c). Nevertheless, it was found that the nanoparticles were capable of penetrating into the cell membranes and causing membrane channels to facilitate penetration of the loaded antimicrobial compounds (Rai et al., 2017). Nanoparticles can also attach to the cell wall, bring about deformity, and eventually result in cell death through high energy transfer (Sharma et al., 2016).

***In vivo* experiments**

Seed germination test

The pure extract seed treatment at the MFC concentration (1.2 mg/mL) and loaded in mesoporous silica nanoparticles led to 100% seed germination, which was slightly higher than that of control water (98 %) but not significantly different (Fig. 4). Nevertheless, compared to control, seed exposure to concentrations of 2.4 and 3.6 mg/mL of the pure extract significantly lowered seed germination by 22.4 % and 60.7 %, respectively, which these concentrations cannot recommend for next tests.

Wheat seeds began to germinate in all treatments 24 hours after sowing except at the concentration of 3.6 mg/L, which delayed germination for 48 hours (Fig. 5). Exposure to 1.2, 2.4, and 3.6 mg/mL pure UDE significantly lowered the speed of germination by 9.6 %, 15.3 %, and 7.4 %, respectively, compared to the control (Fig. 5). In contrast, the usage of UDE-MSNPs combination at 0.6 mg/mL yielded 100 % wheat seed germination and proved to be more effective than pure UDE at 1.2 mg/mL concentration. Indeed, 24 h after sowing, 91 % of treated seeds with UDE- MSNPs, 82 % of empty MSNPs, and 66 % of the pure UDE had germinated compared to 73 % of the controls. These results suggested that seeds loaded in mesoporous silica nanoparticles at 0.6 mg/mL and pure extract at 1.2 mg/mL did not have undesirable effects on wheat seed germination (Fig. 5). The plant compounds can have a positive, negative, or selective effects on seed germination, depending on the concentration of secondary metabolites as well as their interaction with different physiological processes of host plants (Mahmoudi et al., 2020). High concentrations of plant extracts usually lessen the seed germination and low concentrations are similar to controls or slightly stimulator (Khan et al., 2011).

Table 1. Mean (± SE) inhibition percentage of *U. dioica* extract purely and loaded in mesoporous silica nanoparticles against *Gaeumannomyces graminis* var. *tritici* after 10 days.

Treatments	Concentrations (mg/mL)	Inhibition (%)
Pure nettle extract	0.12	8.59 ± 0.41^{e}
	0.39	46.77 ± 0.69^{d}
	0.66	70.11 ± 0.79^{c}
	0.93	82.22 ± 0.35^{b}
	1.2 **	100 ± 0.00^{a}
Loaded nettle extract	0.12	8.66 ± 0.96^{d}
	0.24	42.88 ± 1.34^{c}
	0.36	76.66 ± 0.78^{b}
	0.48 *	100 ± 0.00^{a}
	0.6 **	100 ± 0.00^{a}
Control pathogen (*Ggt*)	-	-

*Minimum inhibition concentration, ** Minimum fungicidal concentration.

Statistics on a completely randomized design were performed using variance analysis (ANOVA). Data were presented as means of five replications. Mean within each column followed by the same letter do not differ significantly based on Duncan's Multiple Range Test at $P = 0.05$.

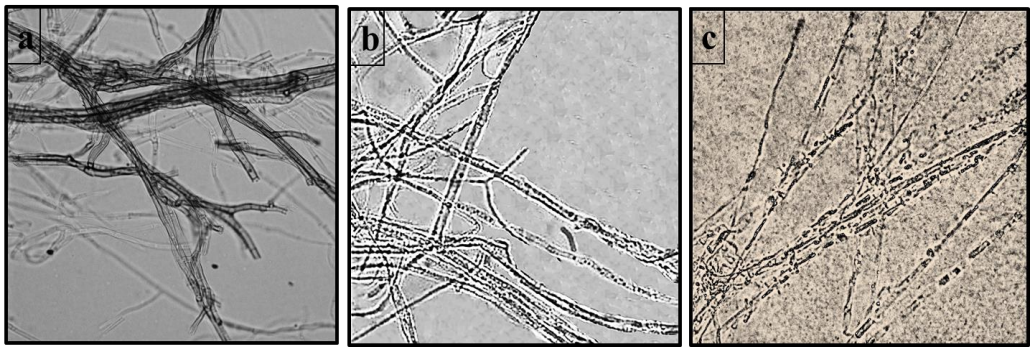


Figure 3. Light microscopy image of a) untreated *Gaeumannomyces graminis* var. *tritici* mycelia, b) treated with pure *Urtica dioica* extract and c) treated with *U. dioica* extract loaded in mesoporous silica nanoparticles. Bar = 10 µm.

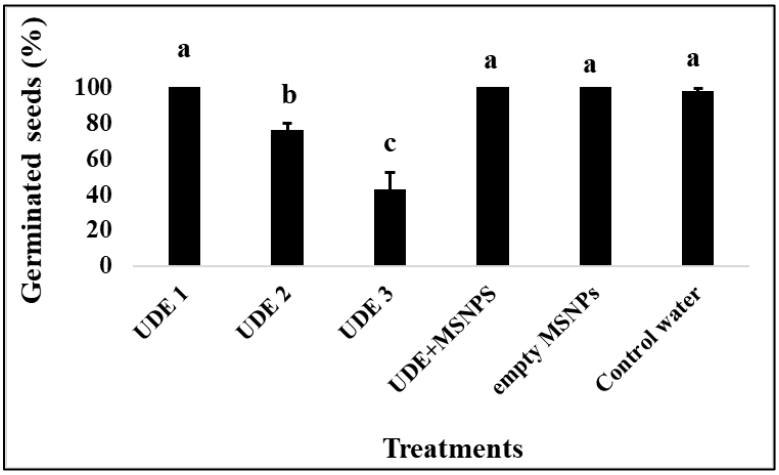


Figure 4. Mean (± SE) germination percentage of treated wheat seeds along nine days. Abbreviations were defined as follows: UDE 1: *Urtica dioica* extract at 1.2 mg/mL (MFC), UDE 2: *U. dioica* extract at 2.4 mg/mL, UDE 3: *Urtica dioica* extract at 3.6 mg/mL, UDE- MSNPs: *U. dioica* extract loaded in mesoporous silica nanoparticles at 0.6 mg/mL (MFC). Mean followed by the same letter do not differ significantly using Duncan's Multiple Range Test at *P* =0.05.

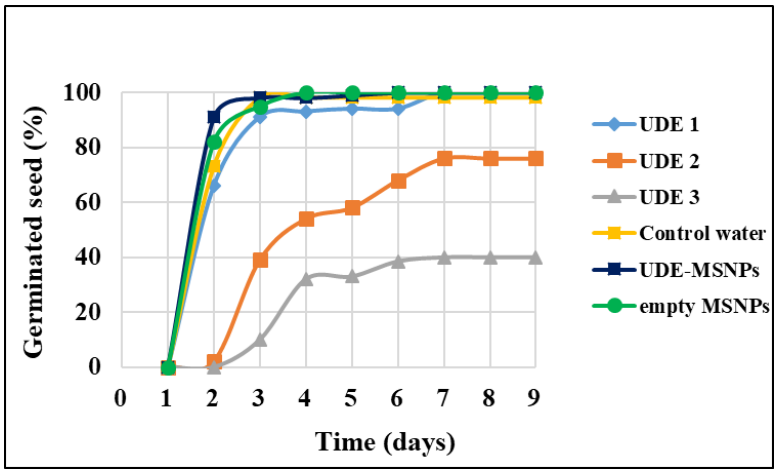


Figure 5. Comparison of effects of different treatments on germination speed of wheat seeds along nine days. Abbreviations were defined as follows: UDE 1: *Urtica dioica* extract at 1.2 mg/mL (MFC), UDE 2: *U. dioica* extract at 2.4 mg/mL, UDE 3: *U. dioica* extract at 3.6 mg/mL, UDE- MSNPs: *U. dioica* extract loaded in mesoporous silica nanoparticles at 0.6 mg/mL (MFC). Data was presented as means of four replications.

The plant compounds can have a positive, negative, or selective effects on seed germination depending on the concentration of secondary metabolites and their interaction with different physiological processes of host plants (Mahmoudi et al., 2020). Elevated seed germination rate has also been proven by cinnamon essential oil activity through nanoparticle encapsulation (Cadena et al., 2018). Silica nanoparticles have a critical role in plant physiology and as hydrated amorphous silica deposited in the endoplasmic reticulum, cell walls, as well as intercellular spaces. Augmented seed germination can be owing to increased silicon uptake by seeds (Siddiqui and Al-Whaibi, 2014). Further, nanoparticles can also open channels in the seed coat and subsequently boost the seeds' water uptake, though the exact mechanism is unclear. Meanwhile, the application of MSNPs to deliver plant natural compounds allows their effective antimicrobial activity at far lower doses (Cadena et al., 2018). The recent property of MSNPs and the stimulant effects of the extract at low concentrations have probably led to the best outcomes in the germination rate of wheat seeds treated with the UDE- MSNPs in this experiment.

Seed and soil treatment by UDE and UDE-MSNPs against *Ggt*

Table 2 outlines the effects of seed and soil treatments on take-all disease severity. Soil application of the MFC of UDE and UDE–MSNPs significantly lowered disease severity, providing high control levels of 78.54% and 80.25%, respectively, compared with the control pathogen. These effects were statistically comparable to soil application of mancozeb (83.9%). The seed treatment with UDE and UDE–MSNPs at MFC also led to effective suppression of *Ggt*, though to a lesser extent than soil treatment, mitigating disease severity by 51.54% and 63.65%, respectively, compared with mancozeb (69.16%). Overall, all treatments significantly alleviated disease severity relative to the pathogen control, with UDE–MSNPs consistently revealing superior efficacy in both application methods. Results of seed and soil treatments on take-all disease severity indicated that soil treatment by UDE and UDE–MSNPs is a suitable method for management of take-all disease.

There is limited information on the antifungal activity of nettle extract or its loaded form against microorganisms (Shabani et al., 2023; Rahmani et al., 2024). The suppressive effects of plant extracts may arise from direct antifungal activity of bioactive compounds such as phenolics, alkaloids, flavonoids, and terpenoids, or through inducing systemic resistance in plants (Abdel-Monaim et al., 2011). Further, sufficient silicon levels in plants are known to boost resistance to pathogens (Solanki et al., 2015). In this study, the MSNPs treatment mitigated the take-all disease by 32.59% in seed treatment and 29.18% in soil treatment, confirming their intrinsic antifungal activity. Similar effects were reported by Derbalah et al. (2018), who attributed MSNP activity to protein inactivation, DNA damage, and cell wall disruption via hydrogen bonding, resulting in membrane destabilization and cell lysis. MSNPs also enhance the solubility of loaded compounds (Le et al., 2019), potentially ameliorating the bioavailability of antifungal agents in the rhizosphere. Finally, silicon can prime phytohormone biosynthesis and signaling, heightening salicylic acid, gibberellic acid, and ethylene levels, and activating plant defense responses following pathogen invasion (Vleesschauwer et al., 2013).

A major limitation of plant pathogens control is the requirement for high application rates of plant extract (Bowers & Locke, 2000). Nanoencapsulation overcomes this constraint, as indicate here, where 0.6 mg/mL UDE–MSNPs were more effective than 1.2 mg/mL pure UDE and yielded results comparable to those of mancozeb.

The effect of UDE and UDE-MSNPs on wheat growth factors

In the soil treatment of inoculated pots, the pure extract, nanoencapsulated extract, and mancozeb revealed an augmented effect on all wheat growth factors compared to the inoculated control (Fig. 6). The application of the pure UDE (1.2 mg/mL) in the absence of *Ggt* has caused a non-significant rise in the shoot length (Fig. 6a), root length (Fig. 6b) plus shoot fresh weight (Fig. 6c), and shoot dry weight (Fig. 6e).

Table 2. Mean (± SE) of *in- vivo* antifungal activities of *Urtica dioica* extract purely and loaded in mesoporous silica nanoparticles as well as mancozeb fungicide against *Gaeumannomyces graminis* var. *tritici*, applied as seed and soil application after 37 days.

Treatments	Seed application		Soil application	
	Disease severity	Control efficacy %	Disease severity	Control efficacy %
UDE (MFC)	2.20 ± 0.12^{c}	51.54	1.00 ± 0.08^{c}	78.54
UDE- MSNPs (MFC)	1.65 ± 0.27^{d}	63.65	0.92 ± 0.29^{c}	80.25
Empty MSNPs**	3.06 ± 0.34^{b}	32.59	3.30 ± 0.36^{b}	29.18
Mancozeb (1gr/1kg seed)	1.40 ± 0.19^{d}	69.16	0.75 ± 0.18^{c}	83.90
Control pathogen (*Ggt*)	4.54 ± 0.20^{a}	-	4.66 ± 0.19^{a}	-

UDE: *U. dioica* extract.
UDE-MSNPs: *U. dioica* extract loaded in mesoporous silica nanoparticles.
**: Empty MSNPs and UDE-MSNPs were applied in equal amounts.
Mean within each column followed by the same letter do not differ significantly using Duncan's Multiple Range Test at $P = 0.05$.

However, it led to a significant increase in the root fresh weight (Fig. 6d) and root dry weight (Fig. 6f) of the wheat seedlings compared to uninoculated control. Further, the increase in the root weight of wheat plants in the soil treated with the pure UDE occurred both in the absence and in the presence of *Ggt;* in the latter case, there was no significant difference with un-inoculated control. However, in *Ggt*-free soil, the usage of the loaded form of the extract at a concentration of 0.6 mg/mL significantly heightened all growth parameters of wheat seedlings compared to un-inoculated control rather than fresh and dry weight of roots.

The UDE has been identified as a rich source of nutrients, minerals, and vitamins (Rutto et al., 2013; Kregiel et al., 2018), which can serve as green manure and boost the crop yield as well as product quality (Shabani et al., 2023). Also, MSNPs protect plants against plant pathogens and enhance crop yields through controlled delivery of bio-fungicide (Rai et al., 2015).

In the seed treatment, pure UDE improved wheat growth parameters in the presence of *Ggt* relative to the pathogen control; however, the impact on the shoot dry weight was not significant (Fig. 7). The most pronounced enhancement of wheat growth,

particularly the root length and fresh weight, was observed with loaded UDE. In the absence of *Ggt*, pure UDE boosted the shoot and root fresh and dry weights, while loaded UDE without pathogen significantly heightened all growth parameters, including the shoot and root length and biomass (Fig. 7). The growth-promoting effects of MSNPs have been previously documented on others plants (Sun et al., 2014) and are attributed to enhanced cellular silica accumulation, an essential element for plant growth (Bao-Shan et al., 2004).

The effect of soil treatment of UDE and UDE-MSNPs on the content of nitrogen

Fig. 8 exhibits the soil treatment effects of pure and loaded extract on the nitrogen content of the aerial parts of the inoculated plants. The data revealed that the amount of nitrogen in the inoculated control (*Ggt*) was significantly reduced compared to the uninoculated control. The application of the pure UDE in the soil at a concentration of 1.2 mg/mL, significantly ($P < 0.01$) boosted the amount of nitrogen in the treated plants compared to the inoculated control, but UDE-MSNPs (at 0.6 mg/mL) presented a higher content of nitrogen than pure UDE did (Fig. 8).

Ggt is a soil-borne pathogen which degrades root cell walls through extracellular enzyme production, thereby impairing nutrient uptake. It disrupts nitrogen absorption through inhibiting nitrogen-assimilating enzymes, especially nitrate reductase, resulting in reduced chlorophyll content and plant biomass (Monsalvez et al., 2010). In the present study, seed and soil treatments with pure UDE and UDE–MSNPs protected wheat plants against *Ggt* infection and significantly boosted growth parameters when compared with the inoculated controls. Notably, in the soil treatments, pure UDE markedly augmented the root fresh and dry weights, resulting in values higher than the inoculated control and comparable to the non-inoculated control under *Ggt* pressure. Further, soil application of UDE–MSNPs in the presence of *Ggt* significantly heightened root biomass beyond both inoculated and non-inoculated controls.

Although scientific evidence is limited, previous studies have noted positive effects of nettle extract on plant biomass. Godlewska et al. (2020) found that 0.1% *U. dioica* leaf extract raised the sprout fresh weight by 113%, while Danilcenko et al. (2017) reported increased seed dry matter following stinging nettle treatments. The effectiveness of UDE and loaded UDE against *Ggt* in pot experiments confirmed laboratory findings, indicating its fungitoxic activity. Nettle extract substantially mitigated take-all symptoms and compensated for pathogen-induced damage, with enhanced efficacy observed for the loaded formulation.

UDE is rich in nutrients, minerals, and vitamins (Rutto et al., 2013; Kregiel et al., 2018) and can serve as green manure, improving crop yield as well as quality (Li, 1994). As a neutrophilic plant, stinging nettle accumulates nitrogenous compounds such as nitrate and free amino acids, and its extract can promote plant growth (Di Virgilio et al., 2015). Accordingly, the stimulation of wheat growth, particularly root biomass in UDE-treated soil, may arise from the combined antifungal activity and nutrient availability, especially nitrogen, promoting root system development and cell wall thickening which limits pathogen penetration. Finally, nanoparticles readily enter plant cells and boost tolerance to biotic stress, while porous nanomaterials improve uptake as well as prolong release of active compounds

(Rastogi & Kandasubramanian, 2019; Shang et al., 2019).

Mancozeb soil and seed applications also effectively controlled take-all disease and promoted growth compared with inoculated controls. Its zinc and manganese content provides essential micronutrients known to contribute to disease suppression as well as plant development (Luo et al., 2020).

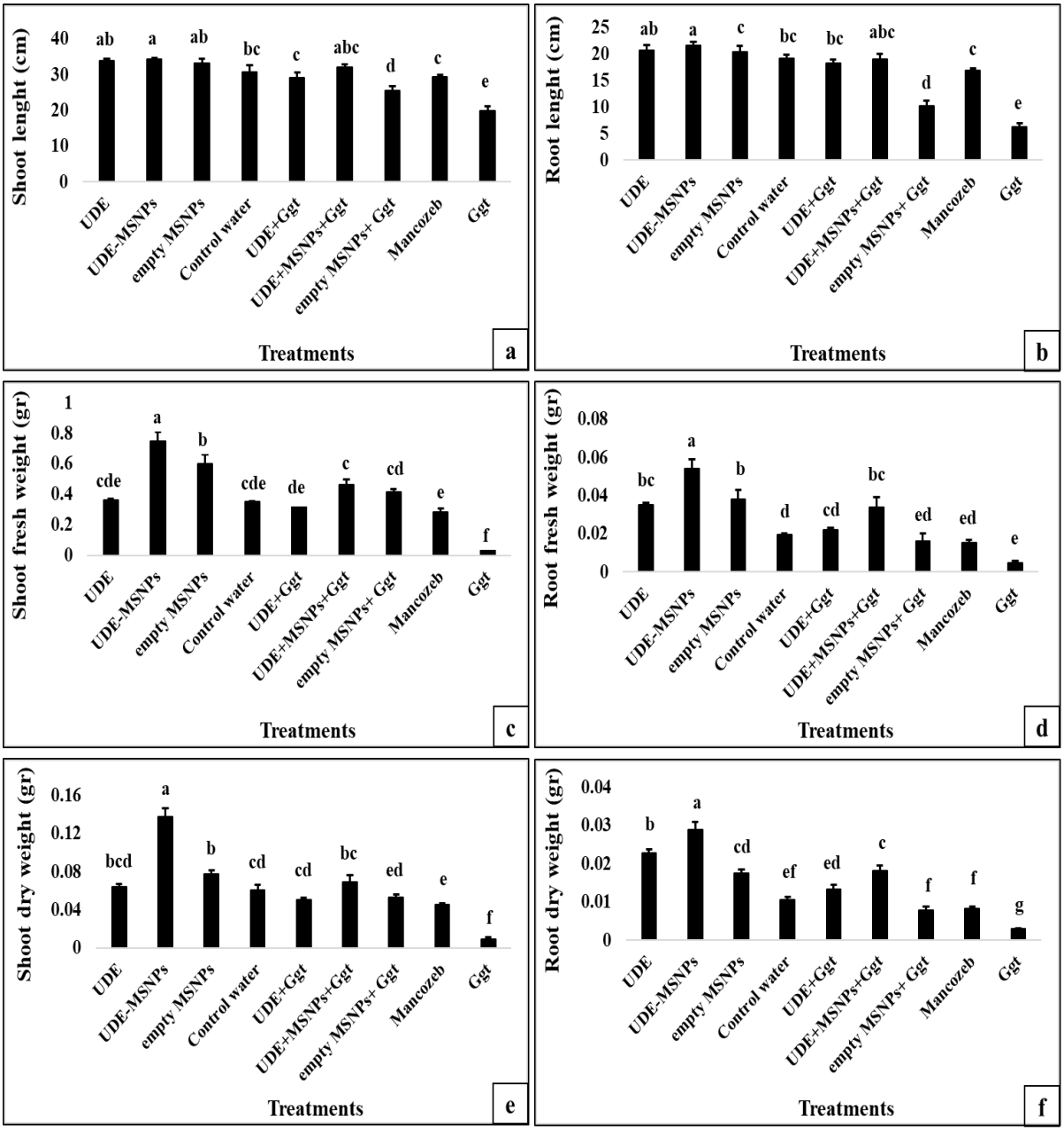


Figure 6. Mean (± SE) of a) shoot length, b) root length, c) shoot fresh weight, d) root fresh weight, e) shoot dry weight and f) root dry weight of wheat seedlings 37 days after soil treatment with *Urtica dioica* pure extract and loaded in mesoporous silica nanoparticles. Mean followed by the same letter do not differ significantly using Duncan's Multiple Range test at $P = 0.05$.

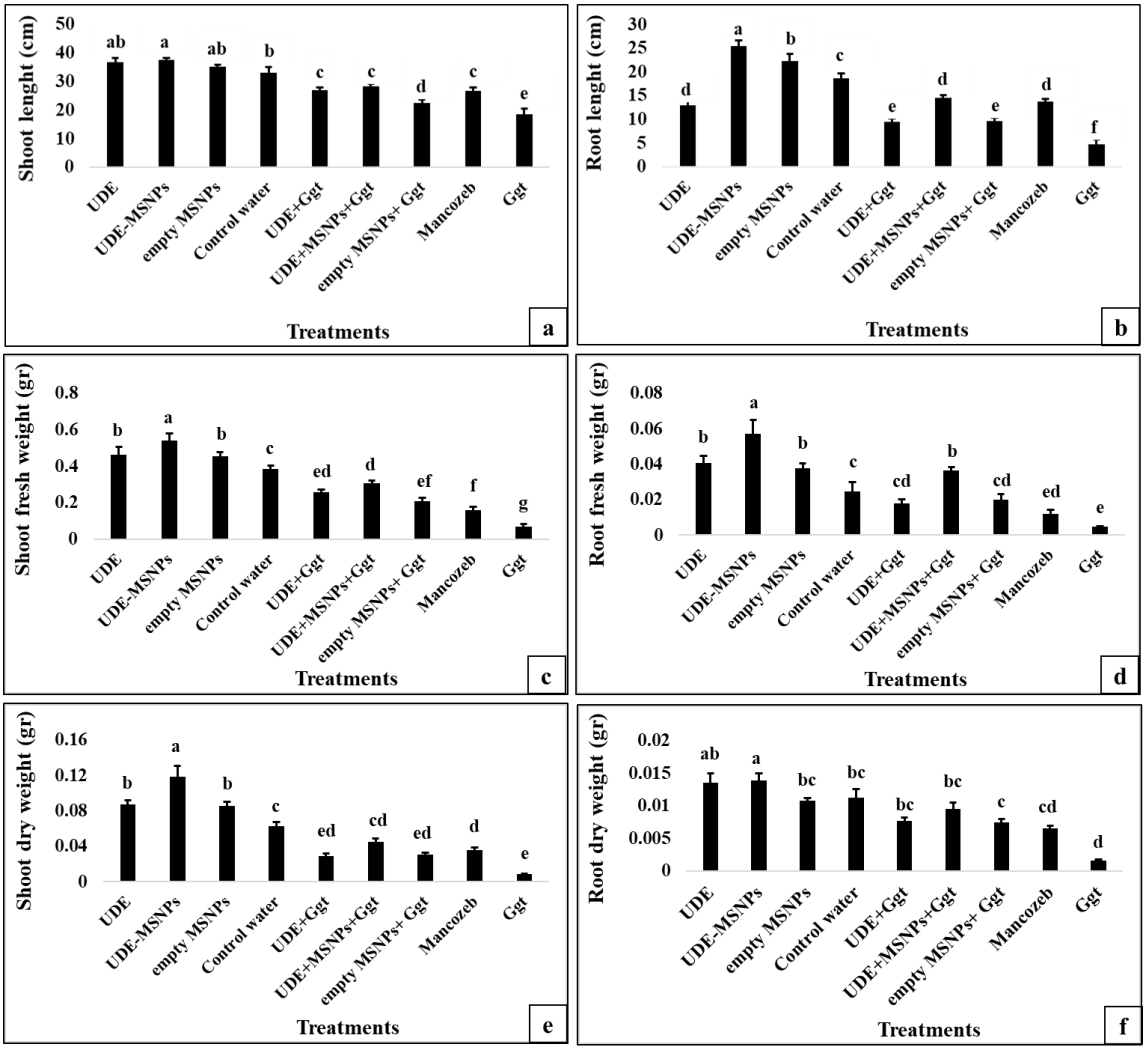


Figure 7. Mean (± SE) of a) shoot length, b) root length, c) shoot fresh weight, d) root fresh weight, e) shoot dry weight and d) root dry weight of wheat seedlings 37 days after seed treatment with *Urtica dioica* pure extract and loaded in mesoporous silica nanoparticles. Mean followed by the same letter do not differ significantly using Duncan's Multiple Range test at *P* = 0.05.

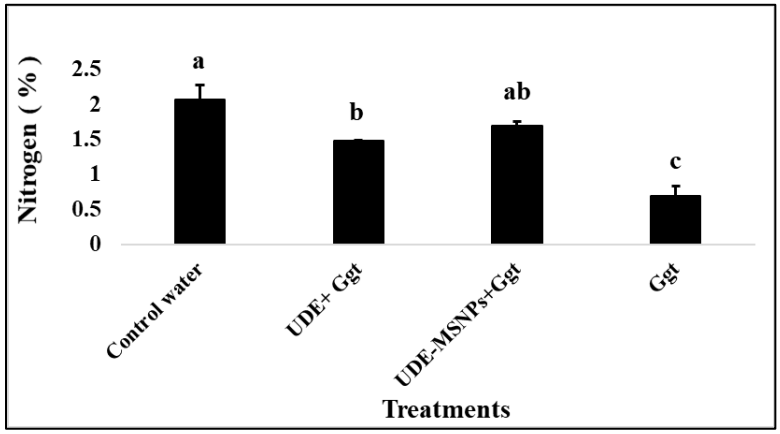


Figure 8. Mean (± SE) content of nitrogen after soil treatment with *Urtica dioica* pure extract and loaded in mesoporous silica nanoparticles. Mean followed by the same letter do not differ significantly using Duncan's Multiple Range test at *P* = 0.05.

Conclusion

Laboratory assays, seed germination tests, and pot experiments indicated that nettle extract is a bioactive antimicrobial compound suitable for eco-friendly management of wheat take-all disease. MSNPs boosted UDE stability and enabled controlled release in soil, augmenting efficacy at lower concentrations and significantly alleviating disease severity of wheat take-all disease. In addition to their intrinsic antifungal properties, silica-based nanoparticles enhance plant tolerance to biotic and abiotic stresses. Thus, UDE–MSNPs represent a promising alternative to synthetic fungicides, though field evaluations are required to confirm their effectiveness.

Acknowledgements

We thank the Nanotechnology Research Center and Plant protection department of the University of Kurdistan (Grant No: Iran Doc, 1411541).

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