Journal of Tropical Crop Science Vol. 13 No. 1, February 2026
www.j-tropical-crops.com

Received 08/09/2025; Revised 13/12/2025; Accepted 08/01/2026
https://doi.org/10.29244/jtcs.13.01.94-102

Potential of *Silybum marianum* Aqueous Extract to Inhibit *Rhizoctonia solani* Growth and Pathogenesis

Saleh H Al-Thabhawi*[1], Firas H Al-Haidary[1], Hussein A Khalaf[2], and Jamal H Kadhim[1]

[1] Department of Plant Protection, Faculty of Agriculture, University of Kufa, Iraq
[2] Department of Plant Protection Techniques, Al-Furat Al-Awsat Technical University, Iraq

*Corresponding author; email: salehh.althabhawi@uokufa.edu.iq

Abstract

The present study evaluated the effects of a cold aqueous extract of milk thistle (*Silybum marianum*) on the growth of the soil-borne pathogen *Rhizoctonia solani* and on wheat seed germination and seedling development. In vitro assays demonstrated that the extract significantly inhibited fungal growth, with concentrations up to 7.5% completely preventing proliferation. Pot experiments further revealed that soil treated with both *R. solani* biomass and its culture medium, particularly in the presence of milk thistle extract, resulted in a drastic reduction in wheat seed germination to 30% and complete seedling mortality within 10 days. Notably, when fungal biomass and its medium were treated with increasing concentrations of milk thistle extract (2.5%–10%), wheat seed germination rates improved markedly, ranging from 83% to 93%. In comparison, seedling death decreased from 16% to 10%. Furthermore, seedlings grown under these conditions exhibited significant increases in shoot and root length, as well as overall biomass, compared with controls grown on potato dextrose agar (PDA) alone. These results suggest that milk thistle extract may serve as an effective pre-planting seed treatment, enhancing wheat germination and seedling vigor while reducing susceptibility to *Rhizoctonia solani* during early developmental stages.

Keywords: phytopathogenic fungi, seed rot, seedlings death, wheat

Introduction

The fungus *Rhizoctonia solani* infects many economically significant crops, causing severe diseases in fields and nurseries. It has a broad family range, estimated at 230 species across 66 families (Ajayi-Oyetunde & Bradley, 2018; Budge et al., 2009). *Rhizoctonia solani* grows over a wide temperature range from 8 to 35 °C (Hamad et al., 2019; Orozco-Avitia et al., 2013). It is one of the soil-endemic fungi that can persist for many years in the absence of a host as stone bodies, resistant to adverse environmental conditions (Gonzáles et al., 2006). On the other hand, most fungicides pollute the environment, are highly toxic to humans and animals, and harm other soil organisms. Given the increasing number of fungal strains resistant to chemical pesticides and the declining efficacy of some of these pesticides, agricultural and research institutions have been prompted to seek alternative solutions using natural products to control plant pathogens (Gwinn, 2018). The use of plant extracts is of great interest to researchers for combating various fungal plant pathogens, as these substances are already present in plants and possess antifungal activity, with desirable characteristics such as acceptable efficiency and rapid decomposition (Choudhury et al., 2018). Extracts of medicinal plants and herbs were used as sources for the production of medicinal drugs or as sources of active ingredients in drug formulations (Choudhury et al., 2024).

Many studies have investigated the effect of plant extracts on the growth of microorganisms, and thus they can be utilized in the treatment of

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various microbial diseases (Kaur et al., 2021). Iraq is home to a large number of wild plants that have significant impact and are of utmost importance when exploited as antifungals and in the manufacture of commercial plant-based pesticides for certain fungal diseases affecting plants. One of these is the milk thistle *Silybum marianum* plant, which is considered a wild medicinal plant and contains some effective compounds, the most important of which are phytosterol, catechin, terpenes, fatty acids, fatty alcohol, and monosaccharide (Eldalawy & Al-Ani, 2020). These substances belong to a series of compounds that have a biological effect on a wide range of organisms (Marceddu et al., 2022).

Milk thistle (*Silybum marianum* L. Gaertn.) is a medicinal and oilseed plant of the Asteraceae family. It is easily identified morphologically by its large spiny leaves with white marbling, erect stems, and purple flower heads (Liava et al., 2023). The species is native to the Mediterranean region. Still, due to its ecological adaptability, high seed production, and tolerance to a wide range of environmental conditions, it has become mostly naturalized in arid and semi-arid regions outside the Mediterranean basin, where it has recently thrived (Moradi et al., 2024). Scientific interest in milk thistle initially arose from its potential as a bioresource for pharmaceuticals and nutraceuticals, and it is increasingly being explored for applications in sustainable agriculture. The seeds contain a flavonolignan complex, silymarin, with antioxidant, hepatoprotective, and antimicrobial activity. The leaves and stems possess phenolic compounds that could have allelopathic effects on neighboring plants (Tsiaousi et al., 2025). Researchers should understand these biological and ecological characteristics of the species in evaluating the potential of the species in agro-ecological systems with such major crops as wheat under different farming practices involving crop rotations; therefore, methodological descriptions must always include details about plant material collection conditions, drying and milling conditions, extraction solvents, and extraction duration, concentration ratios, pH, and preparation method for culture or extract mixtures since these parameters strongly influence both chemical profile-and biological activity-of resultant extracts (Rozhgar et al., 2024). The latest works highlight that it is primarily the incompleteness or vagueness of methodological reporting that prevents experiments from being replicated, thereby emphasizing the need for explicit specification of each procedural step, from sample preparation and storage to germination testing and data analysis (González-García et al., 2025). The authors should also demonstrate that solutions of milk thistle used in seed bioassays have no harmful effect on wheat germination or initial seedling growth. Such proof has been provided: low concentrations (≤1%) aqueous or diluted ethanolic extracts of *S. marianum* display neutral effects, even stimulatory sometimes, on *Triticum aestivum* germination; higher doses result in inhibitory effects, sensitive depending on seeds and extract composition (Lacona et al., 2024; Tsiaousi et al., 2025). Therefore, an explicit concentration range tested, a solvent control type, and statistically significant differences between treated and untreated seeds will assure readers that the milk thistle solution is safe and biologically compatible for use in wheat experiments. Therefore, the study aimed to investigate the in vitro antagonistic activity of the milk thistle plant extract against the phytopathogenic fungus *R. solani*.

Materials and Methods

The Isolation and Propagation of the Fungus *Rhizoctonia solani*

Rhizoctonia solani was isolated from naturally infected wheat (*Triticum aestivum* L.) seedlings exhibiting typical root-rot and crown-infection symptoms, including brown necrotic lesions, tissue maceration, and partial root-system decay. Infected roots and crown tissues were first thoroughly washed under running tap water to remove the soil particles. Then, they were surface-sterilized with 10% sodium hypochlorite (NaOCl) for 3 min, followed by three rinses with sterile distilled water to remove residual disinfectant. Akber et al. (2023)

Received 08/09/2025; Revised 13/12/2025; Accepted 08/01/2026
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recommended this method for efficient surface decontamination of soil-borne pathogens.

Tissues were placed on sterile filter paper and allowed to dry for a few minutes within a laminar airflow. Then, each segment was aseptically cut into approximately 0.5-1.0 cm lengths and transferred to petri dishes (9 cm diameter) containing Potato Dextrose Agar (PDA) medium supplemented with sterile chloramphenicol at 250 mg/L to prevent bacterial contamination (Palacıoğlu et al., 2024). The plates were incubated at 25 ± 2 °C in darkness for 5-7 days until fungal colonies appeared.

Colonies of *Rhizoctonia*-like, fast-growing, cottony white mycelium that develop selectively and turn brown with age, exhibiting right-angled branching, were isolated and purified using the hyphal tip method, which ensures genetic uniformity as described by Nizamani et al. (2025). Pure cultures obtained this way were subjected to morphological identification based on cultural characteristics, both macroscopically and microscopically, following key or descriptions provided by Watanabe (2002), further supported by modern diagnostic references (Akber et al., 2024).

Where applicable, molecular characterization was carried out using the internal transcribed spacer (ITS) region of rDNA to confirm identification, as this is the most reliable genetic marker for differentiating *R. solani* isolates from closely related fungi. The purified isolates were maintained on PDA slants at 4 °C for short-term storage and subcultured periodically to preserve viability and pathogenicity. This integrated approach, initially classical morphology supported by molecular confirmation, represents modern practice in isolating and propagating *R. solani.*

Preparation of Aqueous Extract of Milk Thistle

Samples of milk thistle were collected from agricultural areas in Kufa, brought to the laboratory, air-dried, and then ground to obtain a dry plant powder. The powder was stored in paper bags under laboratory conditions until it was used. The culture medium (stock) was prepared by adding 200 g of the plant powder to 100 ml of distilled water, mixing well, and then completing the volume to 1 L. After 24 hr, the mixture was filtered into another flask using double-layer cheesecloth to obtain the stock extract. Different concentrations of the original stock (0, 25, 50, 75, 100 ml) were prepared for use in the culture medium. (39 g) PDA culture medium was added to the filtrate, and the volume was adjusted to 1 liter to prepare concentrations of 0%, 2.5%, 5%, 7.5%, and 10%. The prepared media were sterilized under vacuum and then poured into petri dishes as required by the experiment. The same media were also prepared without agar to prepare the liquid media.

Effect of Milk Thistle Extract on *Rhizoctonia solani* Radial Growth

Plates of the PDA culture medium treated with the plant extract were inoculated with 0.5 cm diameter discs of 7-day-old Rhizoctonia solani colonies placed in the center of the dish. Three replicates for each concentration were added to the control plates, PDA only. The plates were incubated at 25 ± 2 °C, and the radial growth diameter was measured cumulatively every 24 hr for 3 days. The percentage of inhibition was also calculated using the Abbott equation (1925) and the method of Piepho et al. (2024).

The Effects of Milk Thistle Aqueous Extract on Wheat Seed Germination and Seedling Growth

This experiment was conducted by planting wheat seeds in small plastic cups with a capacity of 250 ml, containing agricultural soil to which the *Rhizoctonia solani* fungus was added, and treated with varying concentrations of a cold aqueous extract of the milk thistle plant. The experiment involved treating the soil in the pots with the contents of culture plates containing the pathogenic fungus *R. solani*, with the entire medium supplemented with milk thistle extract at 0%, 2.5%, 5%, 7.5%, and 10%. Each treatment had three replicates. After 10 days,

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the percentage of seed germination, the number of rotten seeds, the length of the shoot and root system, and the fresh weight of the seedlings were calculated.

Experimental Design and Statistical Analysis

The laboratory experiment was conducted according to a completely randomized design (CRD), while the field experiment followed a randomized complete block design (RCBD) with three replicates. Statistical analysis was carried out using GenStat 12. Statistical analysis was carried out using GenStat 12. The averages were compared using the Least Significant Difference (LSD) test at a 0.05 probability level.

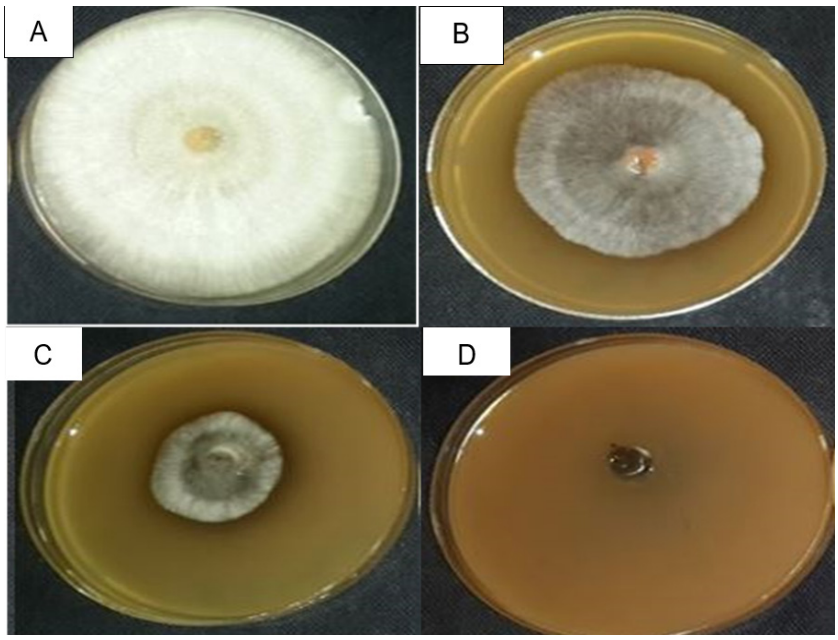
Results and Discussion

Effect of Milk Thistle Extract on *Rhizoctonia solani* Radial Growth

Figure 1 A-D presents the inhibitory effect of cold aqueous milk thistle extract on *Rhizoctonia solani* cultured on potato dextrose agar (PDA). The image displays plates treated with four extract concentrations: 0, 25, 50, and 75 ml/L. In the absence of extract (0 ml/L; Figure 1A), the fungus exhibited unrestricted growth, fully covering the plate. However, introducing 25 ml/L extract (Figure 1B) visibly reduced fungal proliferation. This inhibitory effect became increasingly pronounced at higher concentrations, with 50 ml/L (Figure 1C) and 75 ml/L (Figure 1D) treatments exhibiting a marked decrease in the radial diameter of the fungal colony, reaching minimal growth at 75 ml/L, as detailed in Table 1. Notably, treating the medium with 75 or 100 ml/L extract completely inhibited *R. solani* growth, in contrast to the substantial 8.15 cm radial growth observed in the control (Figure 1A). The percentage inhibition of fungal growth increased from 19.45% at 25 ml/L to total inhibition at 75 or 100 ml/L, as illustrated in Table 1 and Figure 1 A-D. Overall, these results underscore the effectiveness of milk thistle extract, demonstrating a clear inverse relationship between extract concentration and *Rhizoctonia solani* growth.

Figure 1

Rhizoctonia solani Radial Growth on PDA Medium: control (A); Treated with 25 ml/L (B), 50 ml/L(C), or 75 ml/L (D) of Milk Thistle Cold Aqueous Extract



Received 08/09/2025; Revised 13/12/2025; Accepted 08/01/2026
https://doi.org/10.29244/jtcs.13.01.94-102

Effects of Milk Thistle Extract on *Rhizoctonia solani* and its Impact on Wheat Seed Germination and Seedling Development

The results (Table 2) indicate that treating the potting soil with the milk thistle-treated culture medium on which *Rhizoctonia solani* was grown showed an apparent effect on the germination rate of wheat seeds and affected the health of the seedlings with different concentrations of the plant extract. It was found that the highest germination rate (93.33%) and the lowest seedling death rate (10%) were recorded in the treatment with a concentration of 100 ml/L in the presence of the pathogenic fungus with the growth medium components, compared to the control, which recorded a germination rate of 70%, the presence of the pathogenic fungus in the absence of the plant extract reduced the germination rate to 30% and recorded complete seedling death (Table 2).

In the case of the effect of the experimental factors *R. solani* and the milk thistle cold aqueous extract on the vegetative growth indicators, the results (Table 2) indicate that treating the growth medium of the fungus *R. solani* with a concentration of 100 ml of plant extract led to an increase in wheat plant height, root length and the fresh weight of the wheat plants to 15.22 cm and 6.83 cm, and 0.74 g respectively, compared to the uninoculated negative control, which led to 12.06 cm, 1.46 cm, and 0.39 g respectively.

The effect of botanical aqueous extracts on fungal growth has been the focus of numerous studies (Chapagain et al., 2007), which have demonstrated the potential of using plant-derived active ingredients as antifungal agents against plant pathogens, including the widespread

Table 1

Effect of PDA Medium Treated with Milk Thistle Cold Aqueous Extract on Rhizoctonia solani Radial Growth

Milk thistle extract ml/L	*R. solani* radial growth (cm) 72 hr post inoculation	Inhibition rate % over the control
0	8.15	----
25	6.56	19.45
50	3.21	60.52
75	0.00	100.00
LSD (0.05)	0.82	

Table 2

Effects of Milk Thistle Extract on Rhizoctonia solani and its Impact on Wheat Seed Germination and Seedling Development

Milk thistle extract ml/L	10 days post-planting		Growth indicators 21 days post-planting		
	Seed germination %	Seedlings death %	Shoot length (cm)	Root length (cm)	Shoot fresh weight (g)
Control (non-infected)	70.00	10.00	12.06	1.46	0.39
Positive control/infected	30.00	100.00	0.00	0.00	0.00
25	83.33	16.66	13.03	4.00	0.59
50	86.66	13.33	13.22	4.86	0.61
75	90.00	10.00	13.43	5.50	0.62
100	93.33	10.00	15.22	6.83	0.74
LSD (0.05)	17.79	17.79	2.64	1.99	0.23

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Fusarium spp. (Mncube et al., 2019; Kaur et al., 2024) and seed-rot and seedling death caused by fungi (Devi et al., 2024). This confirms the potential of using plant extracts as alternatives to chemical fungicides in controlling and managing plant pests and pathogens (Choudhury et al., 2024). Similar results were obtained by Kursa et al. (2022) on the potential use of plant extracts from various plants as antifungal agents to inhibit the growth of plant-pathogenic fungi, particularly those in the *Fusarium* group. It has been observed that the inhibitory effect of plant extracts is often concentration-dependent, with higher concentrations (not exceeding 20%) showing significant suppression of fungal growth compared to lower concentrations of less than 5% (Hari et al., 2024; Jeewon et al., 2024). For example, milk thistle (*Silybum marianum*) extract was highly effective in reducing *Rhizoctonia solani* growth on treated culture media, while also enhancing seed protection and improving the germination rate of treated Herba polonica seeds (He et al., 2025; Rosinska et al., 2018). The same extract also demonstrated antifungal activity against various *R. solani* isolates from Solanaceae plants (El-Nagar & El-Mohamedy, 2025; Salim et al., 2017).

The underlying mechanism of action is often attributed to the diverse bioactive compounds in these plant extracts, which can act as toxins, enzyme inhibitors, or metabolic disruptors. Such compounds can suppress the fungus's ability to produce secondary metabolites necessary for infection, limiting its parasitic capacity and survival (Al-budairy & Al-Taweel, 2025; Makhuvele et al., 2020; Upadhyay et al., 2015). Moreover, recent research highlights advances in formulation, such as nanoencapsulation, which enhance the stability, bioavailability, and antifungal efficacy of plant extracts under variable environmental conditions (Vakili-Ghartavol, 2025). Overall, these cumulative findings from both classical and recent studies underscore the substantial potential of botanical extracts as eco-friendly, effective, and sustainable tools for managing plant diseases, especially against soil-borne pathogens such as *Fusarium* and *Rhizoctonia* species (Hari et al., 2024; Jeewon et al., 2024).

Conclusions

The findings demonstrated the potential for in vitro inhibition of the pathogenic fungus *Rhizoctonia solani* by treating the fungal growth medium with *S. marianum* extract. Treating the medium with 75 ml/L milk thistle extract completely inhibited fungal growth. Potted soil treated with fungal biomass and its untreated growth medium reduced the germination rate of wheat seeds and leading to complete seedling death after 10 days. However, the presence of milk thistle extract in the fungal-treated medium reduced the pathogenicity of the fungus, allowing germination rates of 83% to 93%, reducing seedling death by more than 80%, and significantly improving seedling growth parameters.

Overall, it can be concluded that milk thistle extract can be used as a pre-planting seed treatment to enhance seedling resistance to fungal infections and improve early-stage plant growth.

It is recommended to explore the use of *Silybum marianum* (milk thistle) extract as a pre-planting seed treatment to enhance wheat seedling resistance against *Rhizoctonia solani*. Integration of milk thistle extract into standard pre-planting protocols can provide an environmentally friendly alternative to chemical fungicides. Regular monitoring of application rates and methods is advised to optimize efficacy and ensure uniform protection across seeds and seedlings.

Acknowledgement

Researcher Salah Al-Dhabawi and co-authors at the University of Kufa, Faculty of Agriculture – Najaf, extend their sincere gratitude to the University President and the Dean of the College for their support and encouragement, which contributed to the successful completion of this research.

Received 08/09/2025; Revised 13/12/2025; Accepted 08/01/2026
https://doi.org/10.29244/jtcs.13.01.94-102

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