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Evaluation of the Inhibitory effect of Ganoderma Extracts against the Pathogenic Fusarium Wilt on Potato Plant

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Abstract:
This study conducted to evaluate the antifungal activity of *Ganoderma lucidum* extracts against *Fusarium oxysporum* isolates, a significant pathogen affecting potato crops. Eighteen *F. oxysporum* isolates were identified and isolated, with three specific isolates' nucleotide sequences registered in the NCBI Gene Bank with accession numbers Mw292595, Mw292596 and Mw292597. The antifungal assays revealed that the *Ganoderma lucidum* culture filtrate, particularly at 50% concentration, significantly inhibited the growth of F. oxysporum isolates F.o1, F.o2, and F.o3 by 53.00%, 44.50%, and 39.30%, respectively. Phenolic and alkaloid extracts derived from the mushroom substrate also exhibited substantial antifungal activity, with the most potent effects observed at a 5% concentration, achieving inhibition rates up to 49.67%. Field trials corroborated the in vitro findings, with the phenol extract treatment improving plant height, tuber mass, and count, indicating the extracts' dual role in pathogen suppression and plant growth promotion. These results conclude that *Ganoderma lucidum* extracts could serve as an effective and sustainable alternative to chemical fungicides in the management of Fusarium wilt in potatoes.

Keywords: *Ganoderma lucidum*, *Fusarium oxysporum*, NCBI Gene Bank, Fusarium wilt

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الخلاصة

أُجريت هذه الدراسة لدراسة الفعالية المضادة للفطريات لمستخلصات فطر الريشي Ganoderma lucidum ضد عزلات فطر Fusarium oxysporum، وهو ممرض مؤثرعلى محصول البطاطا. تم تحديد وعزل ثمانية عشر عزلة من F. oxysporum، وتم تسجيل تسلسلات النيوكليوتيدات الخاصة بعزلتين محددتين في بنك الجينات NCBI. أظهرت اختبارات المضادات الفطرية أن المرشح الزرعي لفطر الريشي، وخاصة بتركيز 50٪، أدى إلى تثبيط نمو عزلات F. oxysporum F.o1 و F.o2 و F.o3 بنسبة 53.00٪ و 44.50٪ و 39.30٪ على التوالي. كما أظهرت المستخلصات الفينولية والقلويدية المستخلصة من المادة الأساسية للفطر فعالية ملحوظة مضادة للفطريات، وكانت الآثار الأكثر فعالية ملاحظة عند تركيز 5٪، محققة معدلات تثبيط تصل إلى 49.67٪. وأكدت التجارب الميدانية النتائج المختبرية، حيث أدى علاج المستخلص الفينولي إلى تحسين ارتفاع النبات وكتلة الدرنات وعددها، مما يشير إلى الدور المزدوج للمستخلصات في كبت الممرضات وتعزيز نمو النبات. تشير هذه النتائج إلى أن مستخلصات فطر الريشي يمكن أن تكون بديلاً فعالاً ومستداماً للمبيدات الفطرية الكيميائية في إدارة ذبول الفوزاريوم في البطاطا.

الكلمات المفتاحية: ***Ganoderma lucidum, Fusarium oxysporum, NCBI Gene Bank, Fusarium wilt***

1. Introduction:

The potato (*Solanum tuberosum* L.) is a cornerstone in global food production, ranking fourth after rice, wheat, and maize (Harris, 1992; FAO, 2008). Its role in addressing the food needs of a growing human population highlights its global significance (Trabelsi et al, 2016). Yet, potatoes face numerous diseases, with many having the potential to damage the crop both pre and post-harvest. Fungal pathogens are a significant concern, inflicting economic losses during growth, storage, and sale (Eken et al. 2000). Among the multiple fungal threats, vascular wilts are prominent. *Fusarium oxysporum* stands out as the primary wilt pathogen, though *Verticillium alboatrum* and *V. tricorpus* are also notable, but less dominant adversaries (Jabnoun-Khiareddine et al, 2005; Daami-Remadi et al, 2011). Globally, it's estimated that Fusarium wilt causal agents, mainly *F. oxysporum*, affect 15 to 70% of potato crops (Thanassoulopoulos and Kitsos, 1985; Gachango, et al 2012; Trabelsi et al, 2016). This rate underscores Fusarium wilt's role as a significant barrier to maximum potato yield (Ommati et al, 2013). The pathogen typically enters potatoes through the roots and affects the stem's vessels. This invasion manifests in various symptoms like leaf necrosis, yellowing, chlorosis, and stunting, which can culminate in the plant's demise (Kucharek et al, 2000; Azil et al, 2021).

Ganoderma lucidum, commonly referred to as lingzhi in China and reishi or mannentake in Japan, holds a revered place in the medical traditions of countries like China and Japan. Its role in enhancing health and extending lifespan has been well-documented in these cultures (Shiao, 2003; Al-Mosawi et al,2021). This mushroom stands out with its large, dark, shiny cap and a unique woody texture. This distinct appearance is vital, as it's primarily the part utilized in ancient remedies. "Lucidum", derived from Latin, means "shiny" or "brilliant", mirroring the mushroom's glossy surface. This study aims to assess the potential of extracts from *Ganoderma lucidum* in inhibiting Fusarium wilt in potatoes. This disease, which plagues potato fields globally, is induced by the Fusarium fungi. Seeking green and sustainable alternatives, our research focused in the possible anti-fungal capabilities of Ganoderma which have known health and medicinal benefits. By testing their effectiveness against Fusarium wilt, our goal is to find an eco-friendly method to combat this prevalent agricultural issue.

2. Materials and Methods

2.1. *Fusarium spp*. Isolation

The fungal pathogen strains were collected from specific regions in spring 2021 (Baghdad and Salah al-Din) governorates , identified by plant leaf yellowing and wilting symptoms the isolates were identified, with three specific isolates' nucleotide sequences registered in the NCBI Gene Bank with accession numbers Mw292595, Mw292596 and Mw292597 (مصدر بحثي الاول)
These strains were isolated directly from potato plant roots. After cleaning the roots with running tap water, they were sectioned into 2 cm segments and immersed in 3% Sodium hypochlorite for two minutes. These segments were then placed on Potato Dextrose Agar (PDA) plates and incubated at 27°C in the dark condition. After a five-day incubation period, emerging fungal colonies were gently recultured to new PDA plates.

2.2. Culturing *Ganoderma lucidum* in Liquid Medium

Ganoderma lucidum was grown in a Potato Dextrose Broth (PDB) medium. 200 ml of PDB was transferred to a 500 ml Erlenmeyer flask and sterilized using an autoclave at 15 psi and 121°C for 20 minutes. Once sterilized, the medium was inoculated with *Ganoderma lucidum.* Freshly harvested mycelial plugs from *Ganoderma lucidum* cultures were added to the flask. The arrangement was incubated at 25°C over 21 days. The *Ganoderma lucidum* culture filtrate was separated from the biomass using Whatman filter paper. For further analyses, the filtrate underwent filtration through a 0.2 Millipore filter membrane (Hadwan et al 1993).

2.3. Assessing the Effect of *Ganoderma lucidum* Culture Filtrates on Pathogenic Mycelial Growth

The antifungal efficacy of *Ganoderma lucidum* culture filtrate, diluted to concentrations of 25% and 50% with sterilized potato dextrose agar, were tested using the poisoned food method (Sultana et al 2007). Agar discs, 5 mm in diameter and covered with pathogen mycelia, were centrally placed on the plates. These plates were incubated at 27°C, with each concentrations tested in triplicate. After 7 days of incubation, the radial growth of mycelium was mesured and compared against a negative control. The inhibitory percentage (L) was derived using the equation:

$$L = [(C - T)/C] \times 100$$

Here, L percentage of inhibition, C signifies the control plate's colony radius, and T the radial expansion of the pathogen when exposed to the extracts.

2.4. Spent *Ganoderma lucidum* Substrate Extracts Preparation

To prepare the alkaloid extract from Ganoderma lucidum's spent mushroom substrate (SMS), 50 g of SMS was immersed in 1000 ml of 80% ethanol for three days at ambient temperature. The mixture was then filtered using Whatman No. 1 filter paper, and the solvent was evaporated under reduced pressure at 50°C using a rotary evaporator.

For the phenolic extract, 50 g of the SMS was submerged in 1000 ml of ethanol and maintained at 60°C for an hour. The extract was then filtered through Whatman No. 1 filter paper and the ethanol was evaporated using a rotary evaporator at 50°C under reduced pressure. The antifungal effectiveness of mushroom extracts at concentrations of 1, 2, and 5% each diluted with sterilized potato dextrose agar was tested using the poisoned food method. Plates were inoculated with 5 mm agar discs carrying the pathogen mycelia, placed centrally. These were incubated at 27°C, and each concentration was replicated three times. After 7 days, the mycelial radial growth was documented and contrasted with a control group. The inhibitory percentage (L) was determined using:

$$L = [(C - T)/C] \times 100$$

In this formula, L is the inhibition percentage, C stands for the colony's radius in the control plate, and T denotes the pathogen's radial growth when exposed to the extracts.

2.5. Evaluation of Chemical Fungicide Tapsin on Pathogenic F1 Strain of *Fusarium oxysporum*

The antifungal capacity of Tapcin fungicide against F1 strain of *Fusarium oxysporum* was studied in vitro using a concentration of 1 gm/L. The poisoned food technique was employed for this assessment, with the methodology mirroring that used for mushroom extracts.

2.6. Field Experimentation

To evaluate the effectiveness of *G. lucidum* extracts, filtrates, and chemical pesticides, a field trial was set up using a randomized block design. The study comprised four treatment groups, replicated thrice, maintaining a 30 cm between plants. Necessary weeding was done, and fertilization was in line with standard potato farming recommendations. Post-plant growth, the plants were treated twice with phenolic extract, alkaloids, *G. lucidum* filtrate, and fungicide. Harvesting was done four months later. Parameters such as infection severity, tuber number and weight and count, wet and dry root weight, branch number , and plant height. The yield was documented, and its percentage variation, relative to the control, was determined.

3. Results

3.1. Fungal Pathogen Isolation and Identification

18 pathogenic isolates were successfully isolated, all of which were identified as members of the Fusarium genus through initial microscopic analysis. Based on the identification methods established by Nelson et al., (1983), these isolates were further classified as *Fusarium oxysporum.* Three specific *Fusarium spp.* isolates were identified and their nucleotide sequences were stored in the NCBI Gene Bank with accession numbers Mw292595, Mw292596 and Mw292597.

3.2. Effect of *Ganoderma lucidum* Culture Filtrates on Fungal Pathogen Growth

The culture filtrate derived from *Ganoderma lucidum* displayed significant antifungal properties against *F. oxysporum* isolates. Specifically, the study showed the filtrate's ability to inhibit the mycelium growth of *F. oxysporum* compared with Tapcin. At a concentration of 50%, it curtailed the growth rate of *F. oxysporum* isolates F.o1, F.o2, and F.o3 by 53.00%, 44.50%, and 39.30%, respectively. In contrast, a 25% concentration didn't exhibit a notable difference in inhibiting Fusarium isolates, indicating that the antifungal potency of *Ganoderma lucidum* filtrate escalates with its concentration.

Table1: Inhibition Ratio of Treatments and Concentrations on PDA at 25°C for 7 days

Treatment/ concentration	Ratio
f.o1-25%	24.47
f.o1-50%	53.00
f.o2-25%	21.53
f.o2-50%	44.50
f.o3-25%	21.53
f.o35-0%	39.30
Fungicide/tapcin1G/L	77.80
Control	0.00
LSD(0.05)	6.87

3.3. Effectiveness of *Ganoderma lucidum* Extracts on *Fusarium oxysporum* Isolates

This research evaluated the efficacy of two Ganoderma mushroom extracts, Phenol and Alkaloid, in comparison to a chemical fungicide, tapcin, on *Fusarium oxysporum* pathogen isolates. Interestingly, all the substances tested displayed antimicrobial capabilities, even at low concentrations. Laboratory results underscored the potential of the phenolic and alkaloid extracts, derived from the spent mushroom substrate of *Ganoderma lucidum*, to impede the growth of *Fusarium oxysporum* isolates at 1% and 2.5% concentrations. Impressively, when applied at a 5% concentration, these extracts exhibited heightened inhibition. To elaborate, the phenolic and alkaloid extracts registered inhibition rates of 43.73%, 49.67%, and 42.67% for F.o1, and 37.10%, 33.40%, and 40.80% for F.o2 and F.o3 isolates respectively.

Table2: Inhibition Ratios by Treatment/Concentration on PDA at 25°C for 7 days

Treatment /concentration	Phenol inhibition ratio%	Alkaloid inhibition ratio%
F.O1-1%	**24.13**	**25.97**
F.O1-2%	**33.40**	**34.50**
F.O1-5%	**37.43**	**43.73**
F.O2-1%	**18.60**	**16.70**
F.O2-2%	**26.00**	**29.51**
F.O2-5%	**33.40**	**49.67**
F.O3-1%	**26.00**	**24.10**
F.O3-2%	**26.15**	**24.13**
F.O3-5%	**40.80**	**42.67**
Fungicide tapcin1G/L	**77.80**	**77.80**
control	**00.0**	**00.0**
LSD 0.05	**8.17**	**5.96**

3.4. Plant Height (cm):

The data from Table (3) delineates the pronounced influence of study variables and their interplay on the plants hight in the F.o1 isolate. Within the treatments, a 5% phenol concentration markedly exhibited the most considerable height of 62.78 cm. Subsequent treatments manifested decreasing plant heights with the alkaloid extract recording 57.00 cm, the pesticide tapcin indicating 50.22 cm, and the mushroom filtrate presenting 46.37 cm. The control, which received no treatment, exhibited the minimum height of 42.11 cm.

3.5. Potato Tuber Mass:

Upon treatment with a 5% phenol concentration, the resultant potato tubers exhibited the maximal mass, registering at 838g. Successively, the treatments of alkaloid extract and mushroom filtrate yielded masses of 553.2g and 533.8g, respectively (Table 3). Tubers arising from the Tapcin pesticide intervention weighed 582.8g, while the untreated controls culminated in the most diminutive tuber masses, approximating 275g.

3.6. Tuber Quantification:

Table (3) the phenol extract treatment manifested an elevated mean tuber count of 18.89. Plants subjected to the mushroom filtrate and pesticide treatments displayed a convergent average of 18.22 tubers. In contrast, the alkaloid extract treatment evidenced a reduced mean tuber count, registering 14.22.

3.7. Dendritic Enumeration:

Table (3) specimens subjected to the phenol extract delineated the paramount average dendritic count, aggregating 5.78. Both the alkaloid extract and mushroom filtrate interventions elicited an indistinguishable mean of 4.33 branches, while the pesticide regimen denoted an average of 4.08 branches.

3.8. Root Mass - Fresh and Desiccated:

Plants intervened with Tapcin showed the zenith of fresh root mass, quantifying 13.62g. The phenol and alkaloid treatments successively reflected fresh weights of 11.86g and 11.73g, overshadowing the mushroom filtrate's 9.7g. In juxtaposition, the untreated control registered the nadir, settling at 8.72g. Concerning the desiccated weight, the phenol extract intervention dominated, measuring 4.23g, while the pesticide Tapcin yielded 3.33g. Marginal disparities were discerned between the dry weights of the alkaloid extract and mushroom filtrate, enumerating 2.48g and 2.69g, respectively.

Table 3: study variables and their interplay on some of agronomic parameters of Potato

Treatment	Plant high (cm)	Potato Tuber Weight (g)	Number of Potato Tubers	Branch Count	Fresh Weight of Root (g)	Dry Weight of Root (g)
Phenol	62.78	838.0	18.89	5.78	11.86	4.23
Alkaloid	57.00	553.2	14.22	4.33	11.73	2.48
Mushroom Filtrate	46.37	533.8	18.22	4.33	9.70	2.69
Pesticide Tapcin	50.22	582.8	17.45	4.08	13.62	3.33
Control	49.33	486.8	14.44	3.89	12.61	3.57
Control with Pathogen	42.11	275.0	6.67	3.48	8.72	3.15
L.S.D 0.05	3.03	56.4	1.00	0.65	0.70	0.41

4. Discussion

It's important to highlight the promising potential of *Ganoderma lucidum* extracts as a sustainable and effective alternative to chemical fungicides for combating Fusarium wilt in potatoes. The isolation and identification of *F. oxysporum* isolates, following classical methods by Gilman, Burnett and Hunter, and Nelson, provide a strong foundation for the study, setting the stage for the exploration of natural fungicides (Gilman 1957; Burnett and Hunter 1972; Nelson et al., 1983).

The substantial inhibitory effect of *Ganoderma lucidum* culture filtrates at a 50% concentration on the growth of *F. oxysporum* isolates is particularly noteworthy. These results align with the known antifungal properties of *Ganoderma lucidum*, as previously documented in the literature (Ganesh

Kumar and Krishnamoorthy, 2014; Priya et al., 2019). The concentration-dependent efficacy suggests that active compounds within the filtrates are likely responsible for this inhibition, supporting the hypothesis that higher concentrations of these bioactive compounds enhance the antifungal effect.

Furthermore, the observed impact of phenolic and alkaloid extracts on plant height and potato tuber mass and count underscores the dual role of these extracts in pathogen suppression and plant growth promotion. The maximal tuber mass achieved with a 5% phenol concentration highlights the potential agronomic benefits of these natural extracts. The findings on dendritic enumeration and root mass further reinforce the multifaceted benefits of Ganoderma lucidum, extending beyond pathogen inhibition to overall plant health. These empirical observations are consistent with the assertions of Ganesh Kumar and Krishnamoorthy (2014), suggesting that the *G. lucidum* mushroom extract exhibits antagonistic activity against pathogens such as *R. solani*, *M. phaseolina*, and *F. oxysporum* lycopersici. Similarly, Priya et al. (2019) identified the inhibitory potential of G. lucidum against the fungal pathogen, *Colletotrichum capsici.* The bio-efficacy of *G. lucidum* is attributed to its comprehensive profile of bioactive compounds, including proteins, sterols, phenols, amino acids, alkaloids, and polysaccharides. Singh et al. (2000) further validate this by underscoring the fungicidal potency of alkaloids derived from *Alstonia venenata*.

The study's insights into the efficacy of natural extracts, particularly when compared to the chemical fungicide tapcin, emphasize the potential for these bioactive compounds to be integrated into agricultural practices. Such integration could reduce reliance on synthetic chemicals, aligning with global trends towards sustainable agriculture. The results warrant further research into the specific mechanisms by which these extracts exert their effects and the exploration of their use in different environmental conditions and crop systems. These findings present a compelling case for the agricultural industry to consider Ganoderma lucidum extracts as a valuable tool in the sustainable management of crop diseases. By offering a natural solution that not only combats pathogen growth but also promotes plant health, these extracts could revolutionize our approach to agricultural pest management.

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