

Ecofriendly Synthesis of Silver Nanoparticles Using Potato Steroidal Alkaloids and Their Activity Against Phytopathogenic Fungi

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ABSTRACT

*In order to reduce the excessive reliance on the toxic chemical fungicides, the present study aimed to isolate the total potato glycoalkaloids (TPAs), and the two steroidal alkaloids α -chaconine and α -solanine from potatoes, *Solanum tuberosum* L. Their structures were characterized using physical and spectroscopic methods including (UV, IR, ¹H, ¹³C--NMR, 2D ¹H-¹H COSY, HMBC and NOESY). Silver nanoparticles (AgNPs) were prepared from potato alkaloids through a green synthesis approach. Potato alkaloids and their nanoparticles inhibited mycelial growth of the phytopathogenic fungi *Alternaria alternate*, *Rhizoctonia solani*, *Botrytis cinerea* and *Fusarium oxysporum* f. sp. *lycopersici* with low minimal inhibitory and minimal fungicidal concentrations. *R. solani* was the most susceptible, while *F. oxysporum* was the most resistant. TPAs was the most fungitoxic (EC_{50} s were 19.8, 22.5, 26.5 and 32.3 μ g/ml against *R. solani*, *A. alternate*, *B. cinerea* and *F. oxysporum* respectively). A mixture of α -solanine and α -chaconine (1:1) showed a marked antifungal activity. AgNPs (size 39.5-80.3 diameter) from alkaloids showed improved fungitoxic activity (EC_{50} s of TPAs nanoparticles ranged between 10.9 and 16.1 μ g/ml). Alkaloids exhibited no or a slight phytotoxicity against wheat and radish. Results recommend the potential of using potato alkaloids and their nanoparticles as biorational alternatives to conventional fungicides.*

Key words: Glycoalkaloids, α -chaconine, α -solanine; nanoparticles, fungicidal activity, phytotoxicity.



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INTRODUCTION

Fungal diseases of plants have always been one of the major problems in crop production causing alterations during developmental stages, severe losses and quality problems related to aspect, shelf life and nutritional values of food commodities ¹. As compared to other plant parasites, fungi cause the greatest impact with regard to diseases and crop production losses ². Control of phytopathogenic fungi is depending primarily on the use of synthetic fungicides. The indiscriminate use of these chemical poisons have led to environmental drawbacks, harmful effects and toxicity hazards to human and non-target organisms, and increased resistant to conventional fungicides ³. Research on alternative, ecofriendly, and economically acceptable control strategies are urgently needed to avoid the adverse effects of agrochemicals used in chemical control. One such alternative strategy is the use of secondary metabolites from plant as biorational tools for pest and disease management to promote sustainable and healthy production of crops ^{4, 5, 6, 7}. Among secondary metabolites involved in the plant defense against fungal pathogens are the plant alkaloids ⁴. Steroidal alkaloids, also known as glycoalkaloids, are secondary metabolites found in numerous solanaceous plants including potatoes (*Solanum tuberosum* L.). These are nitrogenous products synthesized via the triterpene pathway having an oligosaccharide chain attached to the C-3 position of the nitrogenous alkaloid backbone, and are thought to be an important component of the plant's chemical armoury against insects, viruses, bacteria, and fungi ^{8, 9, 10}. In potatoes, the most abundant glycoalkaloids are α -solanine and α -chaconine (Fig. 1) forming as high as 95% of the total glycoalkaloids ⁹. Because of limitations associated with the formulation and application, the performance of plant products, including alkaloids, in crop protection is inadequate and facing major threats. Recently, nanotechnology has emerged as one of the most active areas of research in modern materials science, where synthesis and characterization of nanoparticles gained considerable interest due to their large surface area providing unique chemical and physical properties and potential applications than their bulk counterparts ^{11, 12}. Nanoparticles are more acceptable for various applications due to their high selectivity, sensitivity and their subcellular leading to rapid penetration into living cells ¹³. Metal nanoparticles such as silver, gold and copper have been widely synthesized by employing physical, chemical and biological methods. The bio-based protocols are now turning into viable alternative to conventional physical and chemical methods, as they appear to be simple, low cost and ecofriendly. To avoid the use of risky and toxic solvents, many researchers affirmed the use of biological reducing agents such as bacteria, fungi and plants, that are natural, low-cost, and ecofriendly for producing silver nanoparticles for various nanotechnological applications ¹⁴. The upsurge in green synthesis routes of nanomaterials permit better shape and size control, crystal growth and stabilization of the produced nanomaterials. AgNPs synthesized through green procedures using plant extracts showed antimicrobial activity against pathogenic microorganisms ^{15, 16}, but so far, there has been limited research on the use of AgNPs against plant pathogenic fungi ¹⁷. In the present study, we aimed to determine the antifungal activity of α -chaconine, and α -solanine, two major steroidal alkaloids of potato, when tested individually or as mixtures against *A. alternate*, *R. solani*, *B. cinerea*, and *F. oxysporum* f. sp. *lycopersici*, four of the important pathogenic fungi infecting plants. Silver nanoparticles (AgNPs) were prepared from potato alkaloids through a green procedure and tested as biorational fungicides.

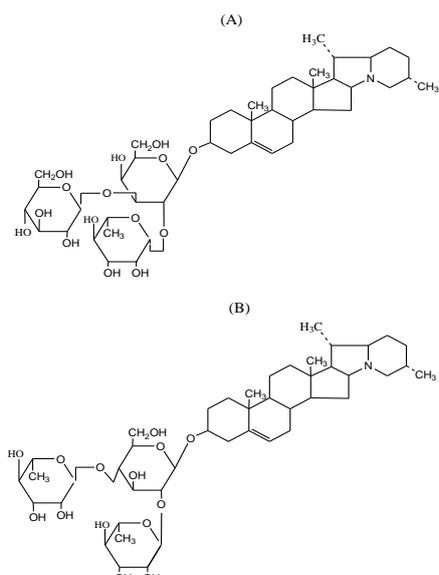


Figure 1. Chemical structures of the major glycoalkaloids of potato (a, α -solanine and b, α -chaconine)

MATERIALS AND METHODS

Fungal Species

A. alternate, *R. solani*, *B. cinerea* and *F. oxysporum* f. sp. *lycopersici* were isolated from potato, tomato and cucumber grown in greenhouses in Najran Province, Saudi Arabia. These fungi are among the most important phytopathogenic fungi in Saudi Arabia. All isolates were grown on potato dextrose agar (PDA; Difco Laboratories, Detroit, MN, USA) at 24°C for up to 14 days.

Authentic chemicals

The glycoalkaloids α -solanine and α -chaconine (label purity ~98.5%) were purchased from Sigma Chemical Company (St. Louis, MO, USA) and used as reference compounds for the extracted steroidal alkaloids. Silver nitrate (analytical grade) was purchased from (Sigma-Aldrich) and used for the preparation of nanoparticles.

Extraction, purification and identification of glycoalkaloids from *S. tuberosum*

Leaves of *S. tuberosum* were collected from local fields at Najran Province, Saudi Arabia. Identification of the plant samples was made by the staff members of Biology Department, Faculty of Arts and Science, Najran University, Saudi Arabia, where a voucher specimen was deposited (voucher number: (St 03). Fresh leaves of the plant were air-dried for 7-10 days in the shade at an environmental temperature (28-32°C daytime). The dried leaves were powdered mechanically by using an electric blender. Five hundred g of the powdered leaves were macerated for 5 days in 95% ethanol (4 L) acidified with 5% acetic acid. The extract was intermittently shaken, filtered, passed over anhydrous sodium sulphate and concentrated under vacuum. The residue was dissolved in boiling water, acidified with 3 ml acetic acid, filtered and rendered alkaline with ammonia 25%. The resultant precipitate (representing the crude glycoalkaloids) was purified by repeated precipitation via adding concentrated ammonia, dried and further purified by crystallization from ethanol, weighed and kept in closed coloured dried bottles^{18, 19}. The resultant crude glycoalkaloid fraction

was fractionated adopting the medium pressure liquid chromatography (MPLC) technique using a silica gel column and a CHCl_3 :MeOH:2% NH_4OH mixture (14:6:1) as a mobile phase^{20, 21}. All fractions obtained from the silica gel column were subjected to a thin layer chromatographic, TLC examination using pre-coated silica gel 60 F254 sheets (Merck, Darmstadt, Germany). The TLC plates were developed in a chamber saturated with 95% ethanol or methanol-chloroform (2:1). The plates were sprayed with aqueous cobalt (II) thiocyanate solution or with sulphuric acid (95%)–methanol solution (1:1), and heated until the spots became visible. The same fractions were pooled, then α -chaconine and α -solanine were washed successively in CHCl_3 and MeOH, crystallized, analyzed by TLC compared with the authentic materials. Structures of the isolated glycoalkaloids were confirmed using spectroscopic instrumentation including UV, IR, ^1H - ^{13}C -NMR, DEPT, 2D ^1H - ^1H COSY, HSQC, HMBC and NOESY.

Green synthesis of silver nanoparticles (AgNPs)

AgNPs were synthesized by the reduction of silver nitrate solution using potato alkaloids²². In Erlenmeyer flask, to 10 ml of the respective alkaloid in hot water, 10 ml of a 1 mM AgNO_3 aqueous solution were added drop-wise under constant stirring at 80 °C within 30–45 min. This solution was incubated in the dark at 37°C (to minimize the photoactivation of AgNO_3). The solution was centrifuged at 4,500 rpm for 15 min after thorough washing and dried at 80 °C for 5– 6 h. Crude pellets were then resuspended in sterile double distilled water, filtered through 0.2 μm filter and stored at 4°C in the dark prior to their use. The color of the resultant solution gradually changed from colorless to greenish brown indicating the formation of AgNPs. Solution with only silver nitrate was maintained at the same conditions and served as a negative control.

Characterization of nanoparticles

Particle size distribution of AgNPs was determined by photon correlation spectroscopy (PCS) at 25°C (HPPS, Malvern Instruments, UK). From the PCS data, the average particle size (z-diameter) was determined by cumulate analysis of the intensity–intensity autocorrelation function²³, and the polydispersity index (PDI), which gives an indication of the width of the droplet size distribution, were determined. Each experiment was performed in triplicate and data were expressed as means \pm S.E.

Preparation of spore suspension and test botanicals

The spore suspension of each fungal species in sterile distilled water was obtained from 5 days old culture and centrifuged. A hemocytometer was used to obtain a homogenous spore suspension of 1×10^8 spores/ml. To prepare the stock solutions of test materials, an appropriate amount of TPAs and individual alkaloids of *S. tuberosum* was dissolved separately in dimethyl sulfoxide (DMSO) to obtain a stock solution of 1 mg/ml and serial dilutions of the stock solution of each compound were made.

In vitro antifungal activity

Antifungal activity of potato alkaloids and their AgNPs against the plant fungi was determined using the agar well diffusion bioassay²⁴. Serial concentrations (0.0156, 0.0313, 0.063, 0.125, 0.25, and 0.50 mg ml⁻¹) of the test compounds were prepared

using DMSO. Test compounds were added to sterilized Potato Dextrose Agar (PDA) (Acumedia Manufacturers, Inc., Lansing, MI, USA) at 40-50 °C and poured into Petri dishes (9 cm diameter). A mycelial disc (5 mm d), taken from the edge of an actively growing colony of each fungi, was placed at the center of each Petri dish. Carbendazim was formulated and tested as a standard chemical fungicide. Control and treated plates were incubated at 28°C for 3 days at the dark, until the growth in the control treatment plates reached the edge of Petri plates. The antifungal index was calculated as (%) mycelial growth inhibition as follows:

Mycelial Growth Inhibition (%) = $[(C-T)/C] \times 100$, where C and T represent the average diameters of fungal growth of control and treatment, respectively ²⁴. The EC₅₀ value (the concentration of each phytochemical that inhibited 50% of mycelial growth) was calculated using probit analysis ²⁵.

The (MIC) and (MBC) of potato alkaloids against the test fungi

A broth two-fold macro dilution method using 96-well microplates was used to determine the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of potato alkaloids and their AgNPs against the test fungi ²⁴. Serial dilutions of each compound and the antifungal standard over the range 0.0039 - 0.50 mg mL⁻¹ were prepared in fungal broth media and ten µL spore suspension (1×10⁸ spores/mL) of each test strain was inoculated in the test tubes using potato dextrose agar (PDA). After inoculation, the microplates were incubated at 28°C for 72 h in the dark. Control groups were made, where fungal spore suspension was inoculated with PDA medium only without any of the tested materials. MIC (the lowest concentration of each tested compound that resulted in no visible mycelial growth after 3 days of incubation) and MFC (the lowest concentration of each tested compound in which no recovery of a microorganism was recorded after 7 days) were recorded.

Phytotoxicity assay

A bioassay based on germination and subsequent radicle and shoot growth was used to evaluate the phytotoxic effects of potato alkaloids against seeds of wheat (*Triticum aestivum* L.) and radish (*Raphanus sativus* L.). To avoid possible inhibition of germination due to fungal or bacterial toxins, seeds were surface sterilized with 15% sodium hypochlorite for 20 min, then rinsed with abundant distilled water, and sown in sterilized Petri dishes (9 cm diameter), containing five layers of Whatman filter paper. Two mL methanol was sprayed on the filter paper in the control group, while 2 mL solution of each alkaloid at assayed concentrations of 50, 100 and 200 µg/ml was poured on the filter paper in the test Petri plates. After complete evaporation of the solvent, ten mature healthy seeds of each plant were placed in each Petri plate and allowed to germinate at 22 ± 2°C with a natural photoperiod. Seed germination was observed directly in Petri dishes, each 24 hours. A seed was considered germinated when the protrusion of the root became evident ²⁶. Each experiment was set up four times along with control. On the fourth day, the effects on percentage germination, root and shoot lengths were recorded.

DATA ANALYSIS

The concentration–response data of antifungal activity were subjected to Probit analysis ²⁵ to estimate the EC₅₀ values and their fiducial limits using the SPSS 23.0 software program (Statistical Package for Social Sciences, Chicago, IL, USA). The EC₅₀ values were considered to be significantly different, if the 95% confidence

limits did not overlap. Data of Phytotoxicity were expressed as (means \pm S.E.). Significance of mean differences between treatments and control were statistically compared using an analysis of variance (ANOVA) at the 5% probability level with individual pairwise comparisons made using Tukey's HSD test.

RESULTS

Concentration of steroidal alkaloids in potato plant

Based on the weight of the dried leaves, the extraction yield (w/w) of the total potato alkaloid extract, TPAs was 70.6%. α -chaconine was the major alkaloid, 35.0% (w/w) and α -solanine content was 28.2%. TLC analyses of compounds revealed the presence of two main spots under UV light (data not shown). Retention indices (R_f) values of α -chaconine and α -solanine were 0.87 and 0.38 mm, respectively. These values were compared well with those of the reference materials and literature.

Formation and characterization of AgNPs

The mean droplet size of AgNPs (expressed as z-diameter) generally comprised between 39.5 nm and 80.3 nm (Table 1). The polydispersity index (PDI) generally comprised between 0.22 and 0.58 nm, indicating a very narrow droplet size distribution. All AgNPs, showed physical stability over one month with neither visible creaming nor significant variation in the mean droplet diameter under refrigerated conditions.

Table 1 - Average size and polydispersity index of AgNPs prepared from potato alkaloids

Alkaloid	*Average size (means nm \pm S.E.)	**PDI
α -solanine (A)	47.0 \pm 5.3	0.28 \pm 0.03
α -chaconine (B)	39.5 \pm 2.5	0.22 \pm 0.05
(A+B)	60.5 \pm 6.4	0.35 \pm 0.06
TPAs	80.3 \pm 7.7	0.58 \pm 0.08

*Values are the mean of three replicates

**polydispersity index

Antifungal activity of potato glycoalkaloids and their AgNPs

Data in Table (2) show that potato alkaloids inhibit mycelial growth of the tested plant fungi depending on the fungal species and the alkaloid tested. In all treatments and regardless of the alkaloid tested, *C. acutatum* was the most susceptible to the test alkaloids, while *F. oxysporum* was the most resistant. TPAs was the most fungitoxic fraction with EC₅₀ values of 19.8, 22.5, 26.5 and 32.3 μ g/ml against *R. solani*, *A. alternate*, *B. cinerea* and *F. oxysporum*, respectively. A mixture of α -solanine and α -chaconine (1:1) showed a marked antifungal activity against all species (EC₅₀ values ranged between 26.4 and 61.5 μ g/ml.). When tested individually, α -chaconine was more fungitoxic than α -solanine against all fungal species. Silver nanoparticles (AgNPs) prepared using potato alkaloids showed improved fungitoxic activity against all species, where EC₅₀ values of AgNPs prepared using TPAs ranged between 10.9 and 16.1 μ g/ml. On the other hand, AgNPs prepared from a mixture of α -chaconine and α -solanine (1:1) showed a pronounced antifungal activity with EC₅₀ values ranged between 18.4 and 22.8 μ g/ml. Fungicidal activity of α -chaconine and α -solanine nanoparticles were also significantly increased (EC₅₀ values ranged

between 26.4 and 49.2 $\mu\text{g/ml}$). As shown in Table (3), antifungal activity of AgNPs prepared using the TPAs fraction was parallel to the commercial carbendazim. The minimal inhibitory concentration (MIC) and minimal fungicidal concentration (MFC) of the test alkaloids ranged between 15.0 and 240 $\mu\text{g/ml}$ (Table 4), where TPAs and a mixture of α -chaconine and α -solanine showed the lowest MIC and MFC values.

Phytotoxic activity of potato glycoalkaloids nanoparticles

A bioassay based on germination and subsequent radicle and shoot growth was used to evaluate the phytotoxic effects of potato alkaloids nanoparticles against seeds of wheat (*Triticum aestivum* L.) and radish (*Raphanus sativus* L.). Data in Table (5) show that the test botanicals exhibited no or slight phytotoxic effect against wheat and radish, where percentage germination and subsequent radical and shoot growth of both plants were slightly affected when treated with TPAs and a mixture of α -chaconine and α -solanine especially at 200 $\mu\text{g/ml}$.

Table 2 - Antifungal activity of potato alkaloids against phytopathogenic fungi using mycelial growth inhibitory technique

Alkaloid	<i>F. oxysporum</i>		<i>B. cinerea</i>		<i>A. alternatae</i>		<i>R. solani</i>	
	EC ₅₀ ^a (f.l. ^b)	slope						
α-solanine (A)	109.7 (99.8-122.0)	1.04 ± 0.14	80.4 (74.1-105.6)	1.01 ± 0.20	71.2 (65.1-84.4)	1.15 ± 0.16	55.0 (48.4-71.2)	1.2 ± 0.16
α-chaconine (B)	80.6 (69.5-95.4)	1.08 ± 0.14	64.1 (55.7-83.6)	1.12 ± 0.12	56.6 (47.7-74.1)	1.22 ± 0.10	43.4 (33.8-56.1)	1.00 ± 0.10
(A+B)	61.5 (48.1-72.3)	0.88 ± 0.08	48.8 (39.3-56.1)	0.94 ± 0.12	36.9 (29.2-50.5)	1.20 ± 0.10	26.4 (21.1-33.0)	0.95 ± 0.08
TPAs	32.3 (27.2-47.9)	0.95 ± 0.08	26.5 (21.1-38.2)	0.92 ± 0.08	22.5 (16.5-30.2)	0.68 ± 0.06	19.8 (14.3-24.2)	0.88 ± 0.08
Carbendazim	15.7 (10.6-19.5)	0.92±0.09	10.8 (8.3-14.9)	1.12 ± 0.12	26.2 (13.1-36.5)	0.72 ± 0.06	12.7 (9.8-17.4)	0.94 ± 0.19

^aThe concentration causing 50% mycelial growth inhibition.^bf.l.= fiducial limits**Table 3** - Antifungal activity of potato alkaloids AgNPs against phytopathogenic fungi using mycelial growth inhibitory technique

Alkaloid	<i>F. oxysporum</i>		<i>B. cinerea</i>		<i>A. alternatae</i>		<i>R. solani</i>	
	EC ₅₀ ^a (f.l. ^b)	slope						
α-solanine (A)	49.2 (43.4-61.1)	0.94 ± 0.10	35.2 (29.1-52.3)	0.94 ± 0.10	40.5 (36.1-50.5)	1.3 ± 0.12	30.0 (26.4-41.1)	1.09 ± 0.08
α-chaconine (B)	38.7 (33.6-48.0)	0.81 ± 0.07	33.2 (24.9-40.5)	0.90 ± 0.10	30.6 (26.7-42.1)	1.00 ± 0.07	26.4 (22.5-34.6)	1.02 ± 0.10
(A+B)	22.8 (18.1-29.3)	0.67 ± 0.06	20.7 (17.3-27.6)	0.84 ± 0.08	18.9 (14.1-26.3)	0.83 ± 0.08	18.4 (14.2-23.3)	0.74 ± 0.08
TPAs	16.1 (9.4-18.8)	0.88 ± 0.09	12.1 (9.3-15.7)	0.74 ± 0.06	10.5 (8.8-14.6)	0.79 ± 0.08	10.9 (5.3-11.7)	0.82 ± 0.06
Carbendazim	15.7 (10.6-19.5)	1.12 ± 0.14	10.8 (8.3-14.9)	0.84 ± 0.08	22.2 (17.3-33.5)	0.94±0.10	12.7 (9.8-17.4)	0.98 ± 0.12

^aThe concentration causing 50% mycelial growth inhibition.^bf.l.= fiducial limits

Table 4 - ^aMIC and ^bMFC values of potato alkaloids and their AgNPs against phytopathogenic fungi

Alkaloid		<i>F. oxysporum</i>		<i>B. cinerea</i>		<i>A. alternatae</i>		<i>R. solani</i>	
		^c MIC	^c MFC	^c MIC	^c MFC	^c MIC	^c MFC	^c MIC	^c MFC
α -solanine (A)	alkaloid	240	120	120	180	90	90	90	60
	AgNPs	120	240	90	120	60	90	60	90
α -chaconine (B)	alkaloid	120	150	120	180	90	120	60	90
	AgNPs	90	120	90	120	60	90	30	30
(A+B)	alkaloid	90	90	90	120	60	90	30	30
	AgNPs	60	60	45	60	30	45	15	30
TPAs	alkaloid	60	60	90	90	30	45	30	30
	AgNPs	30	45	60	60	30	30	15	15
Carbendazim	-	120	120	60	60	30	30	30	30
Solvent	-	NA		NA		NA		NA	NA

^aMIC= minimal inhibitory concentration, ^bMFC= minimal fungicidal concentration

^cValues are the mean of four replicates ($\mu\text{g/ml}$); NA= not active.

Table 5 - *Phytotoxic activity of AgNPs prepared using potato alkaloids against two plant species

Alkaloid	Concentration ($\mu\text{g/ml}$)	<i>Triticum aestivum</i>			<i>Raphanus sativus</i>		
		Germination (%)	RL	SL	Germination (%)	RL	SL
α -solanine (A)	50	100.0 \pm 0.0 ^a	9.02 \pm 0.38 ^a	3.42 \pm 0.16 ^a	90.8 \pm 1.3 ^a	2.40 \pm 0.80 ^a	4.06 \pm 0.58 ^a
	100	96.7 \pm 1.3 ^{ab}	9.0 \pm 0.0 ^a	3.12 \pm 0.12 ^{abc}	86.7 \pm 1.3 ^{ab}	2.32 \pm 0.22 ^a	4.02 \pm 0.61 ^a
	200	93.3 \pm 0.66 ^{ab}	8.7 \pm 0.19 ^{ab}	3.03 \pm 0.11 ^{abc}	83.3 \pm 0.66 ^{abc}	2.18 \pm 0.84 ^a	3.92 \pm 0.42 ^{ab}
α -chaconine (B)	50	96.7 \pm 1.3 ^{ab}	9.02 \pm 0.20 ^a	3.30 \pm 0.15 ^{ab}	90.8 \pm 0.33 ^a	2.38 \pm 0.82 ^a	4.05 \pm 0.54 ^a
	100	96.7 \pm 0.66 ^{ab}	8.62 \pm 0.18 ^{ab}	3.02 \pm 0.09 ^{abc}	86.7 \pm 1.3 ^{ab}	2.26 \pm 0.24 ^a	3.98 \pm 0.60 ^{ab}
	200	93.3 \pm 0.66 ^{ab}	7.88 \pm 0.18 ^{abc}	2.76 \pm 0.15 ^{bc}	83.3 \pm 1.3 ^{abc}	2.22 \pm 0.66 ^a	3.91 \pm 0.53 ^{ab}
(A+B)	50	93.3 \pm 1.3 ^{ab}	8.88 \pm 0.38 ^a	3.35 \pm 0.16 ^a	83.3 \pm 1.3 ^{abc}	2.46 \pm 0.56 ^a	4.03 \pm 0.73 ^a
	100	86.7 \pm 0.66 ^{abc}	8.53 \pm 0.24 ^{ab}	3.03 \pm 0.12 ^{abc}	80.0 \pm 1.3 ^{abc}	2.30 \pm 0.52 ^a	3.91 \pm 0.39 ^{ab}
	200	83.3 \pm 0.33 ^{bc}	7.63 \pm 0.19 ^{abc}	2.88 \pm 0.15 ^{abc}	80.0 \pm 0.66 ^{abc}	2.11 \pm 0.24 ^{ab}	3.85 \pm 0.41 ^{ab}
TPAs	50	90.8 \pm 0.0 ^{abc}	8.58 \pm 0.38 ^{ab}	3.31 \pm 0.13 ^{ab}	80.0 \pm 0.0 ^{abc}	2.26 \pm 0.56 ^a	4.02 \pm 0.55 ^a
	100	83.3 \pm 0.33 ^{bc}	8.14 \pm 0.24 ^{abc}	3.20 \pm 0.12 ^{ab}	76.7 \pm 0.33 ^{bc}	2.08 \pm 0.52 ^{ab}	3.80 \pm 0.62 ^b
	200	76.7 \pm 0.33 ^c	7.3 \pm 0.19 ^c	2.58 \pm 0.12 ^c	73.3 \pm 0.66 ^c	1.64 \pm 0.24 ^b	3.52 \pm 0.51 ^c
Control	-	96.7 \pm 1.1 ^{ab}	9.04 \pm 0.28 ^{ab}	3.34 \pm 0.15 ^{ab}	90.8 \pm 1.6 ^a	2.44 \pm 0.83 ^a	4.06 \pm 0.68 ^a
** F values	-	12.2	7.2	4.6	6.6	4.2	12.05

*Values are the mean \pm S.E. of 4 replicates; RL=Radicle growth (length of seeds, cm); SL=Shoot length (cm).

Means within the same column followed by the same letter(s) are not significantly different ($P \leq 0.05$) (Tukey's HSD test).

**All F-values are significant at $P \leq 0.001$.

DISCUSSION

Percentage of alkaloids extracted from *S. tuberosum* are similar to previous reports^{8, 19, 27}. Little variations in the abundant of these alkaloids were observed, which could be attributed to differences in stages of growth, environmental and genetic variations, nutritional status of the plant, and the extraction conditions²⁸. Steroidal alkaloids of potato exhibited considerable fungicide activities against four of the major pathogenic fungi attacking plants with a slight phytotoxicity when applied as a spray to etiolated 4-day-old seedlings of wheat and radish. In the literature, a number of plant alkaloids, including steroidal alkaloids, have been reported to possess antifungal activity against plant pathogens^{8, 10, 29}. Comparing our results with those reported earlier, α -solanine and α -chaconine of potato showed antifungal activity against *Ascobolus crenulatus*, *Alternaria brassicicola*, *Phoma medicaginis* and *Rhizoctonia solani*²⁹. In all cases, α -chaconine was more inhibitor than α -solanine. Similar findings were reported when the antifungal activity of five steroidal alkaloids were tested against *B. cinerea*¹⁰.

As secondary metabolites, steroidal alkaloids are not involved in the essential pathways of plants. These substances are considered as natural toxins protecting the plant against fungi, insect pests and other herbivores^{8, 30}. Their biological activities are largely due to their membrane-disruptive effects by complexation with the membrane 3β -hydroxysterols ultimately to form aggregates and cause loss of membrane integrity^{31, 32, 33}. Their toxicity could also based on their anticholinesterase activity on the central nervous system, and to changes caused in active transport of ions through membranes, resulting in disorders in general body metabolism. It was found that the tetrasaccharide moiety of the glycoalkaloid was crucial for toxicity because of its membranolytic action^{29, 33}. Usually chacotriose-based glycoalkaloids are highly active, whereas solatriose-based compounds show lower or no membrane-disruptive activity^{19, 27, 32, 33}. Results of the present study are in accordance with these findings, where α -chaconine was more fungitoxic and phytotoxic than α -solanine. However, experiments examining the effects of plant defenses against pathogens often examine individual defenses in isolation, despite evidence that many defense mechanisms are additive or synergistic³⁴.

According to our results, a mixture (1:1) of α -chaconine and α -solanine, or the total potato alkaloid fraction (TPAs) showed higher fungitoxic activity, indicating an additive and/or synergistic manner in which these secondary metabolites exert their biological effects. These results are in a good accordance with those of Fewell and Roddick²⁹ who stated that a mixture of α -solanine and α -chaconine (1:1) produced marked synergistic antifungal effects against *A. crenulatus*, *A. brassicicola*, *P. medicaginis* and *R. solani*, meanwhile individual alkaloids showed lower activity. Similar findings were reported when the effect of berberine and santonin, two alkaloids isolated from rhizomes of *Berberis aristata* and flower buds of *Artemisia maritima*, respectively were assessed against some saprophytic and obligate fungi, where mixtures of both alkaloids found to be more effective than individual ones³⁵. The same conclusion was reported when the activity of α -solanine and α -chaconine as natural insecticides was evaluated against stored grain coleopteran pests^{19, 27}.

Results of the current study indicated that silver nanoparticles AgNPs (size 39.5 to 80.3 nm) prepared from potato alkaloids showed increased fungicidal activity against the test phytopathogenic fungi. Many of the fungicides known today are organic compounds with poor water solubility. Development of nanoproducts appear to solve this problem through enhancing water solubility of poorly water-soluble substances, resulting in improvement of their biological activities. Their bioavailability resulted also in stable formulations without utilization of organic toxic solvents¹³. In the literature, green synthesized AgNPs using plant extracts showed promising fungicidal

activity against plant pathogenic fungi. Examples are AgNPs from *Acalypha indica* leaf extract against *A. alternata*, *Sclerotinia sclerotiorum*, *Macrophomina phaseolina*, *R. solani*, *B. cinerea* and *Curvularia lunata* ³⁶, nanosilver suspension from raspberry extract at 50 ppm against *Cladosporium cladosporoides* and *A. niger* ³⁷, AgNPs synthesized using turnip leaf extract against the wood-degrading fungi *Gloeophyllum abietinum*, *G. trabeum*, *Chaetomium globosum*, and *Phanerochaete sordida* ³⁸, AgNPs biosynthesized using *Tridax procumbens* L. extract at 50 ppm against *A. flavus* and *A. niger* ³⁹ and AgNPs from *Aloe vera* leaf extract against the two pathogenic fungus *Rhizopus sp.* and *Aspergillus sp.* ¹⁴.

Although, fungicidal activity of extracts and natural products from several plant species against phytopathogenic fungi have been well-confirmed, fungicidal activity of potato steroidal alkaloids, especially at their nanoscale reported herein is considered a first report. The ongoing increase in the health hazards and environmental problems with chemical fungicides make the use of botanicals more attractive for combating harmful pathogens. The greener nanotechnology approach with natural products-based nanomaterials looks very promising and could be adopted pragmatically to protect crops from fungal attack.

Reports on the mechanism of antimicrobial activity of AgNPs have shown that silver ions (Ag^+) interact strongly with the thiol (-SH) group of membrane bound proteins or the lipid bilayer that destabilizes the membrane, causing ion leakage and cell rupture ⁴⁰. Ag^+ affects also the function of membrane-bound enzymes, such as those in the respiratory chain, causing destruction of membrane integrity ^{41, 42}. Upon treatment with Ag^+ , DNA loses its ability to replicate resulting in inactivated expression of ribosomal subunit proteins ⁴³. Certain cellular proteins and enzymes essential to ATP production are also altered upon treatment with silver ions ⁴⁴. Concerning their effects on plants, AgNPs could interact with intracellular parts of the plant tissues causing water imbalances, cell damage, and decreases in photosynthesis ⁴⁵. They are also reported to have genotoxic effects on plant cells, inducing chromosomal aberrations and micronucleus induction ⁴⁶, and DNA damage and lipid peroxidation ⁴⁷. Nevertheless, the lack of knowledge on the possible damage due to the low discharge levels of nanomaterials, has led to a gap in our understanding of the behavior and fate of nanomaterials in the environment ⁴⁸. Therefore, their potential hazards to biological systems and their interactions with organisms should be understood. Consideration of such interactions is necessary for choosing and designing nanomaterials with minimum adverse impacts on the the environment and human health.

CONCLUSIONS

Based on the results of the present study, potato steroidal alkaloids and their green synthesized nanoparticles showed significant fungicidal activity against phytopathogenic fungi with low phytotoxic properties. These secondary metabolites could be lead to rational design of new fungicides with new or selective mode of action and minimal side effects on non-target organisms including users.

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