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Sugarcane Bagasse Wastes Represent Untraditional Pillars for Antifungal Silica-based Nanoparticle and ß-Glucosidase Production

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Abstract

As a natural raw waste of agricultural origin, sugarcane bagasse has several potential future applications upon converting into promising products. Both alkaline-treated solution and cellulose pulp were used for preparation of antifungal silica-based nanoparticles (Si-NPs) beside glucose and production. Adopting the agar-disc diffusion technique, nanosilica successfully exhibited in vitro antifungal activity towards the pathogen *Fusarium solani*, inhibition zones of 0.4-0.9 mm dia. were measured. In a pot experiment, increasing levels of NPK fertilization regimes and Si-NPs were examined for protecting potatoes from the fungal attack. The highest rates of both amendments supported sufficient tuber yield and vegetative growth, plants heavily dressed hosted 9-25 tubers plant^{-1} with fresh weights of 159.9-550.6 g plant^{-1}. The pathogenicity of *F. solani* towards the vegetable plant significantly diminished in presence of NPK and Si-NPs. Tuber biomasses positively correlated with NPK doses (r, 0.918) and Si-NP rates (r, 1.00), except the low level of 25 % that failed to promoted growth and yield even in presence of full NPK regime. *A. niger* succeeded to convert cellulose pulp into reducing sugars and ß. glucosidase. The enzyme production significantly optimized after 12 hr. incubation at 200 rpm particularly in culture medium amended with 3 % corn speed liquor. Interestingly, adding 5 ml drop-wise distilled water to β. glucosidase production medium seemed necessary to avoid the feedback inhibition of the enzyme.

Keywords: Sugarcane bagasse; *Aspergillus niger*; *Fusarium solani*; Silica-nanoparticle; Saccharification; ß. glucosidase; potato.

1. Introduction

The last few decades witnessed a great progress in the adoption of nanoscience and technology for the development of a vast array of materials for application in the agricultural sector. Indirectly, such materials conspicuously authenticated unlimited ability to ameliorate the quality and quantity of food commodities. Actually, nanosubstances possess extraordinary/exceptionally novel characteristics encompassing a wide surface-area-to volume ratio and high reactivity beside enhanced catalytic and biological properties. These special merits put nanomaterials on the map of the appropriates for a variety of applications in agriculture and biomedicine. To date, numerous nanomaterials have been developed in addition to many more others are currently under production, application and evaluation. It is well established that a great number of nanoparticles have successfully been synthesized via physical, chemical and biological strategies [1]. Unfortunately, the conventional physical and chemical procedures applied for nanoparticle synthesis might result in the formation of toxic substances and generate wastes of injurious impacts on the environment [2]. To overcome this miserable situation, replacing by different biological agents such as plants and microbes together with their products has recently gained considerable attention worldwide due to rapid

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synthesis in addition to the eco-friendly nature of these materials. Apart from plant materials, fungal members as biological agents have been preferably used for the synthesis of several nanoparticles. Generally speaking, nanoparticle synthesis by fungi is referred as mycosynthesis [3, 4]. The advantages of using filamentous fungal candidates over other bioagents like bacteria [5]. These obviously include the exceptional tolerance towards toxic heavy metals, easier cultivation of biomass, in addition to the fact that nanoparticle synthesis is extracellular which reduces the costs of down streaming, etc. Among the fungal genera, the different species of Fusarium are considering the prime choice and commonly used by scientists. Several species such as *F. acuminatum*, *F. culmorum*, *F. oxysporum*, *F. semitectumand* and *F. solani* [6, 7] have been successfully used for synthesis of nanoparticles like gold, palladium, platinum, silica, silver and others. All of these fungal species are having the ability to synthesis nanoparticles with better size and monodispersity [8].

Of the nanoparticles synthesized by Fusarium spp., Si-based nanoparticles (Si-NPs) have been reported as a unique Si source that is necessary element for plant growth and known by its role to support the resistance under rigorous environmental conditions. Soil-applied Si-NPs seemed more effective than those foliar-applied [9]. As well, Si-NPs significantly improved the development and oil content of Cymbopogon citrates [3]. Seed priming and soaking in Si-NPs markedly increased shoot and root length of *Helianthus annuus* seedlings.

Sugarcane bagasse is among the harmful wastes to the environment if kept without treatment because it contains cellulose, hemicelluloses, lignin, polyposis besides few levels of extractive and mineral compounds. After being treated with KOH, the bagasse bundles started to dismantle and the fiber detached from other components. This alkaline treatment results in the swelling of the biomass following by increasing the internal surface area of the lingo cellulose particles, in addition to weakening of the structural integrity of the lingo cellulose and breaking off the bond linkages between lignin and both cellulose and hemicelluloses causing greater accessibility and digestibility of the cellulose fraction, and thus, it could be depolymerized into fermentable sugars [10]. From the resulting solution after KOH treatment, silicon could be precipitated.

Potato (*Solanum tuberosum* L.) is ranking the first among the non-cereal food crops for human consumption and has the potential to ensure food security. Therefore, the satisfactory productivity and nutrition quality improvement is the major target for potato producers. Minimization of yield losses due to pests, diseases and environmental stresses besides better post-harvest management must be considered as well. More than 40 pathogens including viruses, bacteria, fungi, nematodes and insects are causing damage to foliar parts and tubers [11]. Worldwide, about 13 Fusarium species are responsible for dry rot disease in potato [12]. Apart from being pathogenic, Fusarium members are established to produce a number of mycotoxins in food products; sambutoxin, trichothecene, fusarin C, fusaric acid, zearalenone and deoxynivalenol [13].

The present piece of work is among the on-going research attempts in the area of nano-biotechnological applications for plant development and human welfare. It comprised: 1) securing the silicon element from the alkaline-treated solution of sugarcane bagasse, 2) transformation of silcon into nanoparticles by mechanical blending, 3) assessment of antifungal potential of silica-based nanoparticles (Si-NPs) towards the pathogen *Fusarium solani* adopting agar-disk diffusion method, 4) production of glucose and ß -glucosidase enzyme from the cellulose pulp of sugarcane bagasse and 5) biomanagement of root rot disease in potato infested with *F. solani* via Si-NPs integrated with NPK fertilization.

2. Materials and Methods

2.1. Sugarcane bagasse recycled by-products

Representative samples of NaOH-treated solution and cellulose pulp of sugarcane bagasse were kindly provided by Edfo Company for Paper Manufacture. Samples were collected in clean black glass bottles.

2.2. Separation of silica from alkaline-treated solution

An aliquot of 10 ml H_2SO_4 (10 %) was drop-wise added to 1000 ml alkaline-treated solution to adjust its initial pH of 13.1 to 7.0. Thereafter, the solution mixture was kept for 24 hr. at ambient temperature to allow the precipitation of silica. The produced silica gel was thoroughly washed in distilled water to get rid of the other main constituents of the plant material then air-dried.

2.3. Preparation of silicon-based nanoparticles (Si-NPs)

Silica-based nanoparticles were prepared adopting the modified procedure of [14]. The dried silica gel, as pellets, was mechanically blended in metal container for 15 min. to convert into powder. Representative powder samples were checked by scanning electron microscopy to guarantee their sizes as nanoparticles. For the preparation of different concentrations of the produced Si-NPs, a portion of 100 g of powder was suspended in 1000 ml distilled water to represent the full level of the product. From which, the concentrations of 75, 50 and 25 % were prepared. For the pot experiment, the various levels of Si-NPs were uniformly distributed on soil surface.

2.4. Characterization of Silica nanoparticles (NPs)

Formation of silica NPs was firstly observed by visual color. Further UV-Vis spectroscopy, and TEM techniques were applied in the Research Park of the Faculty of Agriculture, Cairo University. Silica NPs obtained were characterized independently by UV–Vis spectra recorded at room temperature using Instrument "Thermo Scientific HERYIOS ٢, USA" for their surface Plasmon Resonance (SPR) ranging from 200 to 800 nm [15]. UV–Vis analysis is highly common for primary detection of different kinds of NPs with the capability to absorb electromagnetic radiation in the UV–Vis spectral region.

Morphology and size of NPs were examined by transmission electron microscopy (TEM) onto an amorphous carbon coated copper grid, dried and analyzed using a (Jeol JEM 2100 TEM, Tokyo, Japan) operated at voltage of 80 kV, as descriped by [16].

2.5. Production of sugars and ß-glucosidase from cellulose pulp

The collected cellulose pulp was washed several times then immersed in distilled water for 10 min and its initial pH of 9.2 was adjusted to 7.0 by H_2SO_4 10 %, rewashed in distilled water and air-dried. The dried material was mechanically blended for 10 min for the uniformity of the produced small pieces. Three different culture media recommended for ß-glucosidase production by *A. niger* using the procedure described by [17, 18, 19] were compared among each other after 3 hr. incubation at 37 ºC and 100 rpm. The first comprised (g L^{-1}) of: cellobiose, 2.5; peptone, 0.75; $(NH_4)_2SO_4$, 1.4; $CaCl_2$, 0.3; $MgSO_4.7H_2O$, 0.3; KH_2PO_4, 2.0, in addition to (mg L^{-1}) of: $FeSO_4.7H_2O$, 5.0; $CoCl_2$, 2.0; $MnSO_4.7H_2O$, 1.6 and $ZnSO_4.H_2O$, 1.4. The second consists (g L^{-1}) of: xylan, 3.0; peptone, 5.0; yeast extract, 1.0; glycerol, 2.5; urea, 0.5; $MgSO_4.7H_2O$, 0.5; $NaNO_3$, 0.5; KH_2PO_4, 2.0 and KCl, 0.5. The third medium contained (g L^{-1}): glucose, 10.0; corn steep liquor, 7.5; $MgSO_4.7H_2O$, 0.1; $NaNO_3$, 1.0; K_2HPO_4, 0.3; KCl, 0.1 and $FeSO_4.7H_2O$, 0.01.

The carbon source of the most appropriate medium for enzyme production was replaced by cellulose for saccharification by *A. niger* according to [20]. A portion of 50 mg cellulose was mixed with one ml acetate buffer (0.1 M, pH 4.8) and one ml of the fungus culture filtrate. The mixture was incubated at 50 °C for 24 h and the released reducing sugars were determined. Saccharification percentage was calculated by the following equation:

Saccharification (%) = 0.9 x reducing sugars (mg) / 50 mg pretreated cellulose x 100

In an attempt to guarantee an appropriate ß-glucosidase enzyme production, *A. niger* was allowed to grow in the culture medium and exposed to various cultivation conditions. These conditions are limited to incubation periods of 1, 2, 3, 4, and 5 days as well as shaking at 50, 100, 150 and 200 rpm, in addition to various concentrations of corn steep liquor (0.50, 0.75, 1.00, 2.00, 3.00 and 4.00).

The enzyme activity was determined by incubating one ml of diluted enzyme in one ml silica in (1 %) in acetate buffer (0.1 M, pH 4.5) at 50 °C for 30 min and the reducing sugars produced were measured. β-glucosidase involves in the hydrolysis of β (1-4) glucosidic linkages of disaccharides *e.g.*, cellobiose, oligosaccharides and glucose-substituted molecules, for hydrolytic activity (HA) assay, 50 mg microcrystalline cellulose (MCC) was suspended in one ml acetate buffer (0.1 M, pH 4.8) and one ml of the fungal culture filtrate. The HA is expressed as mg reducing sugars/50 mg MCC/24 hr.

For possible large scale production of reducing sugars and glucose by ß-glucosidase on the industrial level, a simulating double-jacket system (Fig. 1) was constructed where the column was partially filled with 1 g cellulose together with 100 U of the enzyme. At 50 ºC incubation, 1-hr. periodical samples of the products were taken along a period extended to 12 hr.

To avoid the expected feedback inhibition of the enzyme whenever the products accumulate, distilled water drops in a total of either 5 or 10 ml volume were gently added using dilling flow and the final products

were collected and measured.

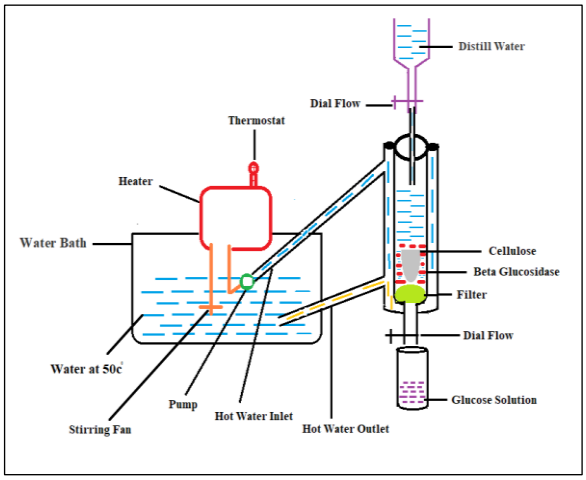

Fig. 1. Simplified sketch for the constructed double-jacket system for saccharification of cellulose by ß -glucosidase of *A. niger*.

2.6. Soil

Soil used for the pot experiment was taken from the Agricultural Experimental Station at Ismailia governorate, air-dried, crushed and sieved to pass 2 mm screen. The soil is sandy in texture with the following characteristics: organic matter, 0.54 %; total nitrogen, 1.9 %; pH, 7.72 and EC, 0.20 dSm^{-1} [21]. Soil was distributed in plastic pots of 30 cm dia. and 30 cm depth at the rate of 8 kg pot^{-1}.

2.7. Plant material

Tubers of potato cv. Spounta were supplied by Potato Brown Root Project, Agricultural Research Centre (ARC), Giza. Tubers were selected to be similar in size and weight as possible, thoroughly washed in tap water and bud-hosted tuber pieces were cut and allowed to germinate for 48 hr. on moistened cotton in glass Petri dishes. Developed seedlings were distributed 3 cm sub-surface soil as 4 pot^{-1}.

2.8. Mineral fertilization regime

The NPK fertilization regime of ammonium sulfate (20.6 % N), super phosphate (P_2O_5, 15.5 %) and potassium sulfate (K_2SO_4, 50 %) was applied at the recommended rates for potato cultivation equivalent to 700, 300 and 250 kg fed^{-1} respectively. The pot experimental design comprised as well the incorporation into soil the NPK levels representing 25, 50 and 75 % of the recommended quantities. The fertilizers were mixed well with soil prior to tuber seedling distribution.

2.9. Fungal strains tested

Aspergillus niger strain NRRL- 3 in addition to the pathogen *Fusarium solani* strain NRRL-22163 known by its pathogenicity towards potatoes were obtained from Northern Regional Research Laboratory (NRRL). A loopful from 5-day old fungal culture on PDA medium was transferred to 100 ml medium, gently shaken, and incubation took place for 5 days at 30 °C. For soil infestation with the pathogen, 100 ml pathogen liquid culture were added over-head soil. For comparison, the fungicide ROOTOLEX was uniformly spread on soil surface at the recommended

2.10. Antifungal potential of silica-nanoparticles

The susceptibility of *Fusarium solani* and *Aspergillus niger* to Si-NPs was assayed by the agar-disc diffusion method [22]. Petri dishes containing PDA medium were inoculated with the fungus. Different concentrations of Si-NPs (25, 35, 50, 75 and 100 %) were introduced in holes on agar plate surface. Plates were incubated at 30 °C for 3-5 days and the fungus inhibition zone diameters were measured in mm.

2.11. Potato-pathogen-NPK- silica nanoparticle interweave

Three-month pot experiment was executed at the greenhouse of the Agricultural Research Center, Giza to expound how far the pathogen-NPK-silica nanoparticle interactions might affect the development and yield of potato. The experimental layout consisted of 22 treatments (Table 1) encompassing all the possible variable interweaves. Pots were arranged in a complete randomized design with three replications. Potting soils were irrigated with tap water when needed to maintain *ca*. 60 % WHC.

At the 90th day, plants were carefully uprooted, separated into shoots and roots and the tubers were gently collected. The plant materials were determined for a number of agronomic traits. Those included length, fresh and dry weight of shoots and roots besides number and fresh weight of produced tubers. In addition, indole acetic acid (IAA) and absesic acid (ABA) contents in roots and shoots were estimated adopting the procedures of [23].

2.12. Statistical analyses

Based on [24] data were subjected to one-way ANOVA analysis and treatment means were compared by the least significant difference at p (0.05). Linear regressions and correlation coefficients among different variables were illustrated and calculated as well.

3. Results

3.1. Scanning electron microscopy (SEM) examination of silica-based nanoparticles (Si-NPs)

The Si-NPs were successfully synthesized by the applied mechanical and precipitation methods. The SEM images of the produced nanoparticles are depicted in (Fig. 2). It is clearly shown that they have somewhat spherical structure with diameters falling in the range of 23.2-35.4 nm. The images indicate, as well, a high purity level of the produced material. Such extremely small sizes of Si-NPs permit a satisfactory attachment to any other partner, represented in the present study by the examined pathogenic fungus *Fusarium solani*. A finding that obviously alleviate/restrict its pathogenicity towards the host.

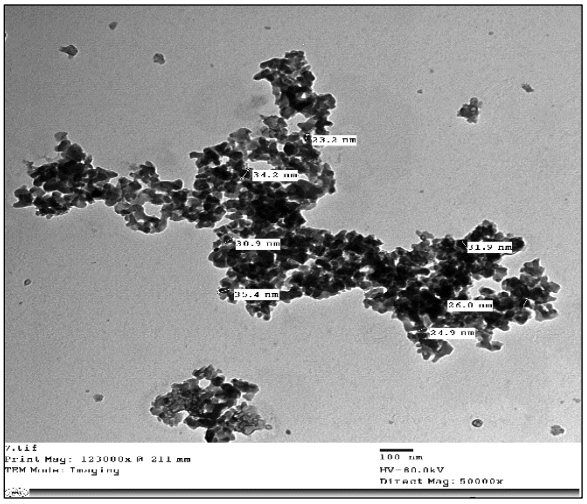

Fig. 2. SEM image of silica-based nanoparticles prepared from the alkaline-treated solution of sugarcane bagasse waste.

Table 1. Layout of the greenhouse experiment

Potato Tr. No.	*Fusarium solani*	Rootolex	NPK* 100	NPK* 75	NPK* 50	NPK* 25	Si-NP** 100	Si-NP** 75	Si-NP** 50	Si-NP** 25
1	-	-	-	-	-	-	-	-	-	-
2	-	-	●	-	-	-	-	-	-	-
3	-	-	-	-	-	-	●	-	-	-
4	●	-	-	-	-	-	-	-	-	-
5	●	●	●	-	-	-	-	-	-	-
6	●	-	●	-	-	-	-	-	-	-
7	●	-	●	-	-	-	●	-	-	-
8	●	-	●	-	-	-	-	●	-	-
9	●	-	●	-	-	-	-	-	●	-
10	●	-	●	●	-	-	-	-	-	●
11	●	-	-	●	-	-	●	-	-	-
12	●	-	-	●	-	-	-	●	-	-
13	●	-	-	●	-	-	-	-	●	-
14	●	-	-	-	-	-	-	-	-	●
15	●	-	-	-	●	-	●	-	-	-
16	●	-	-	-	●	-	-	●	-	-
17	●	-	-	-	●	-	-	-	●	-
18	●	-	-	-	●	-	-	-	-	●
19	●	-	-	-	-	●	●	-	-	-
20	●	-	-	-	-	●	-	●	-	-
21	●	-	-	-	-	●	-	-	●	-
22	●	-	-		-	●	-	-	-	●

*, mineral nitrogen fertilizer rates related to the recommended levels of 100 %

**, the various concentrations of silica nanoparticles related to the initially prepared quantity of 100 %.

3.2. Synthesis of nanosilica *from alkaline black liquor substance*

The conversion of 1000 ml of alkaline black liquor into 90 g nanosilica was recorded and the appearance of nanosilica are in (Fig. 3) which found the shape of the particles as a powder with amorphous form and limited amount of particles are agglomerated with white color.

Fig. 3. Nano silica powder

UV-Vis absorption spectrum of these solutions were screened at wave length range 200-800 nm, and exhibits a distinct absorption peak (Fig. 4) in the region of 229.5-294 nm, strong peak was appeared at 285 nm. Moreover, in the visible range at 400-800 nm not found any peaks [25].

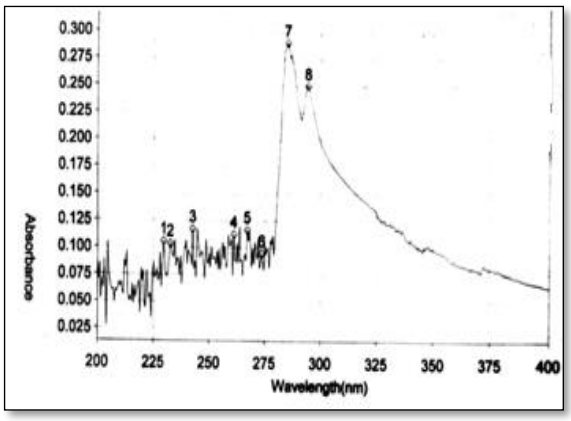

Fig. 4. UV-Vis spectrum of Nano silica

3.3. Antifungal potentials of silica-based nanoparticles

The detrimental impacts of silica-based nanoparticles on *A. niger* and *F. solani* are shown in (Fig. 5). For both fungal members, raising the Si-NP level proportionally inhibited the growth, the pattern of inhibition was strain-dependent. The lowest nanoparticle quantity of 25 % had no apparent influence on fungi, the level of 35 % slightly injured both microbes. The highest depressive effects were observed at the level of 100 %. In general, *F. solani* seemed more susceptible for the applied nanoparticles than *A. niger*, the average inhibition zones were 0.63 cm for the former and 0.36 cm for the latter.

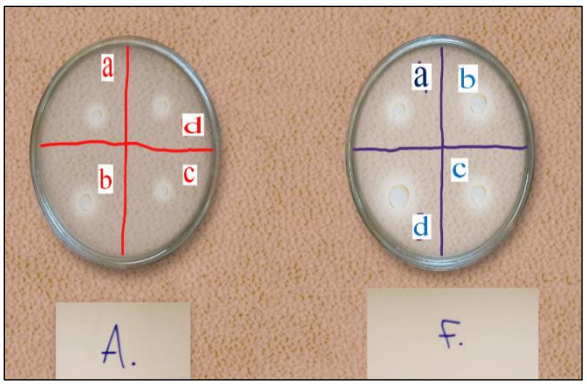

Fig. 5. Antifungal activities of silica-based nanoparticles towards A. niger (A) and F. solani (F); a, b, c, d; nanoparticle levels of 35, 50, 75, 100 % respectively.

3.4. Saccharification of cellulose and ß -glucosidase production by A. niger as affected by cultural media composition

The capability of *A. niger* to produce sugars and ß -glucosidase enzyme was compared among three of the recommended *growth* media of different carbon sources. The culture medium containing xylan and glycerol [19] was not that favourable for the enzyme synthesis where the lowest amount of 2.9 IU/ml was recorded (Table 2). The enzyme yield significantly increased to 11.8 IU/ml in presence of glucose and corn steep liquor in the cultivation medium [20]. An appropriate yield of ß -glucosidase of 7.5 IU/ml was estimated in culture medium supplemented with cellobiose [18]. Substitution of cellulose instead of glucose resulted in the superior production level of the enzyme (16.3 IU/ml). The hydrolytic potential measurements behaved in trends similar to those of the enzyme production. Reducing sugar contents were falling in the range 0.09-0.50 mg ml^{-1} being the highest in case of cellulose-enriched culture medium. The inferior yield of glucose (0.06 mg ml^{-1}) was obtained in xylan and glycerol-received culture medium, against as high as 0.34 mg ml^{-1} when cellulose was used as carbon source.

Table 2. Comparative effect of culture media on sugar and ß-glycosidase production by *A. niger*

Culture media*	ß-glycosidase (IU/ml)	Hydrolytic potential Reducing sugars (mg ml^{-1})	Glucose (mg ml^{-1})
[17]	7.5	0.23	0.16
[18]	2.9	0.09	0.06
[19]	11.8	0.36	0.25
Cellulose-replaced	16.3	0.50	0.34
LSD (0.05)	13.8	0.08	0.02
CV (%)	13.4	5.05	8.11

*, refer to materials and methods.

3.5. Impacts of cultivation conditions on ß -glucosidase production and hydrolytic potential of A. niger

(Table 3) speaks well on to what extent cultivation conditions might govern the capability of the fungus *A. niger* to produce sugars and ß -glucosidase from cellulose. In respect to incubation period, prolonged time up to 120 hr. deemed necessary to achieve the maximum enzyme yield of 105.0 IU/ml, this reflected as well on both reducing sugars (3.18 mg ml^{-1}) and glucose (2.17 mg ml^{-1}) contents. Somewhat shorter incubation time of 96 hr. resulted in almost similar enzyme yield of 103.1 IU/ml as well as reducing sugars (3.12 mg ml^{-1}) and glucose (2.13 mg ml^{-1}) quantities.

When the fungus allowed to grow for 120 hr. in cellulose-containing culture medium and exposed to various shaking regimes, both ß -glucosidase and hydrolytic potential significantly affected. While the recommended shaking rate of 100 rpm was sufficient to secure proper enzyme yield (103.1 IU/ml), elevating the culture growth speed to 200 rpm recorded *ca*. 11 % increased enzyme amount. The highest reducing sugars (3.46 mg ml^{-1}) and glucose (2.36 mg ml^{-1}) contents were estimated for 200 rpm-shaken fungal culture medium.

As a natural N-rich organic waste, the corn steep liquor (CSL) was evaluated as a nutritive substrate for improving sugar and ß -glucosidase production in the *A. niger* culture medium containing cellulose and incubated for 120 hr. at 200 rpm. The enzyme pool steady increased as the concentration of CSL increased.

Table 3. ß-glycosidase production and hydrolytic potential of *A. niger* as affected by cultivation conditions

Parameters	ß-glycosidase (IU/ml)	Hydrolytic potential Reducing sugars (mg ml^{-1})	Glucose (mg ml^{-1})
Incubation period (hr.)			
24	17.5	0.53	0.36
48	33.7	1.02	0.70
72	72.7	2.21	1.50
96	103.1	3.12	2.13
120	105.0	3.18	2.17
LSD (0.05)	13.6	0.44	0.29
CV (%)	2.9	9.3	4.8
Shaking rate (rpm)			
50	58.0	1.75	1.20
100	103.1	3.12	2.13
150	113.7	3.44	2.35
200	114.2	3.46	2.36
LSD (0.05)	10.1	1.01	0.77
CV (%)	6.4	8.6	5.5
Corn steep liquor (%)			
0.50	14.1	0.43	0.29
0.75	16.3	0.49	0.34
1.00	32.1	0.97	0.66
2.00	47.5	1.44	0.98
3.00	72.7	2.21	1.50
4.00	69.3	2.00	1.43
LSD (0.05)	19.4	0.49	0.46
CV (%)	6.6	9.1	2.9

In comparison with the routinely used quantity of CSL (0.75 %), raising the culture medium content from the waste to 3 % resulted in an extraordinary increase of more than 300 % in the produced enzyme amount. This supportive influence extended as well to either reducing sugars or glucose content.

Generally, the relationships between ß -glucosidase production and either incubation time, shaking rate or CSL concentration are expressed in the calculated positive correlation coefficients (Rs) of 0.97, 0.87 and 0.96 as well as linear regressions illustrated (Fig. 4).

3.6. Alleviation of ß -glucosidase feedback inhibition for sustainable enzyme production

The possible feedback inhibition of ß -glucosidase due to saccharification of cellulose and the accumulation of sugars produced was monitored along a fermentation period extended to 12 hr. This necessitated measuring the products at 1-hr. intervals up to the point of hydrolytic activity of the enzyme decreased, following the convey of water flow to the fermentation medium to prevent the product accumulation and sustainable enzyme production.

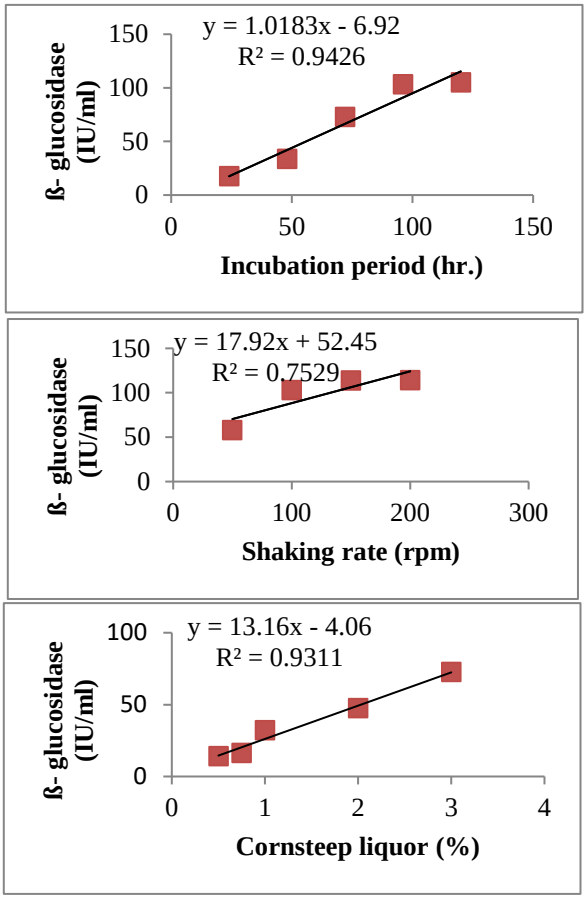

Fig. 4. Linear regressions and coefficient of determination (R2) among ß-glucosidase yield and the various cultivation conditions.

The enzyme hydrolytic potential expressed as reducing sugars and glucose formation steady increased up to 3 days, thereafter, the activity decreased. At that moment, 5 ml distilled water were drop-wise added to prevent the accumulation of the products, this resulted in a storm of hydrolytic potential of the enzyme reaching 154.8 and 105.9 mg ml^{-1} of reducing sugars and glucose respectively. Prolonged fermentation time severely decreased the enzyme activity and the product quantities (Table 4).

The previous experimental scenario was repeated but the added distilled water increased to 10 ml. No apparent differences were reported in respect to hydrolytic potential of ß -glucosidase, where the 3rd day of fermentation period was the switch-off point of enzyme feedback inhibition.

The drop-wise introduction of distilled water, at the rate of 5 ml, was compared to one-shot addition into fermentation medium for optimized enzyme activity. Results in (Table 4) indicate almost akin behaviour of ß -glucosidase in both with relatively lower product yields recorded for the latter strategy.

3.7. Potato development in soil infested with F. solani in presence of Si-NPs and NPK fertilizers

Growth attributes of the 90th old potato plants cultivated in sand soil infested by the pathogenic fungus *F. solani* simultaneously received various silica-based nanoparticles and NPK fertilization regimes considerably varied among the applied treatments. Plants grown in bare soil poorly developed and appeared dwarf being 5 cm in length. Soil infestation with the pathogen dramatically affected plant growth and resulted in 20 % reduction in length compared to untreated ones (data not shown). In absence of NPK fertilization, 100 % Si-NPs application significantly compensated the deleterious impact of the pathogen. The situation markedly modified when the nanoparticles were added in conjugation with the mineral fertilizers, up to 400 % increase over untreated plants was recorded.

The productive yield of tubers speaks much better on the influence of the applied treatments (Table 5). Plenty of tubers (27 $plant^{-1}$) were formed by the full NPK-dressed plants. As high as 11-23 tubers $plant^{-1}$ were produced by those received the full NPK fertilization regime in combination with Si-NPs whatever the dose. Numbers decreased by *ca*. 39 % in average when the NPK application level decreased to 75 %. Among the NPK/Si-NP treated potatoes, those supplemented with 25 % NPK in presence of 25 % nanoparticles hosted 7 tubers $plant^{-1}$ being the inferior. The unamended *F. solani*-infested plants harbored only 4 tubers $plant^{-1}$. The tuber biomass yield is one of the reliable parameters to justify the direct effect of a given treatment on vegetable crops. The full NPK-fertilized potatoes produced the heaviest tubers compared to all the other experimented ones, this is indicated by the highest fresh weight of 590.8 g $plant^{-1}$. When the NPK levels decreased, even in presence of Si-NPs, the fresh weights gradually decreased. Regarding the effect of Si-NP application dose, 75 % together with mineral fertilizer seemed the superior particularly in case of 100 % NPK (501.7 g $plant^{-1}$). The combined treatment of full dose of both NPK and Si-NPs successfully guaranteed appreciable tuber biomass of 489.3 g $plant^{-1}$. Potatoes infested by the pathogenic fungus showed the lowest biomass of 93.1 g $plant^{-1}$. In general, and apart from nanosilica amount, raising the quantities of NPK increased tuber fresh weights. This is expressed in the linear regression illustrated in (Fig. 5-a) and calculated correlation coefficient of 0.918 Regardless the NPK application

rates, the linear regression in (Fig. 5-b) indicates that tuber fresh weights proportionally increased as the level of Si-NPs increased up to 75 % with estimated correlation coefficient of 1.00 nanosilica at 25 % was not that supportive to tuber biomass yield.

Table 4. Hydrolytic potential (mg ml^{-1}) of ß -glucosidase in fermentation medium received distilled water to avoid the expected feedback inhibition of the enzyme.

Fermentation period (hr.)	Drop-wise water addition (5 ml)		(10 ml)		One-shot water addition (5 ml)	
	Reducing sugars	Glucose	Reducing sugars	Glucose	Reducing sugars	Glucose
1	29.4	20.3	29.4	19.4	26.2	18.2
2	45.9	32.6	45.9	30.2	41.4	29.4
3	51.3	35.4	51.3	34.1	45.6	33.1
4	89.7	61.7	89.7	60.1	83.3	59.8
5	95.6	65.3	95.6	62.7	85.2	61.4
6	108.1	74.3	108.1	71.9	93.8	70.1
7	114.2	78.6	114.2	75.2	101.2	73.5
8	123.2	84.5	123.2	82.6	103.4	79.6
9	131.7	90.1	131.7	89.7	112.6	88.8
10	154.8	105.9	154.8	104.4	122.3	102.3
11	75.2	52.2	75.2	51.9	59.7	51.6
12	25.9	18.4	25.9	17.2	18.1	16.2

Table 5. Potato growth and yield attributes in soil infested by *Fusarium solani* and received NPK fertilizers and silica-based nanoparticles.

Treatment No.*	Tubers (per plant)		Shoots (g plant^{-1})		Roots (g plant^{-1})		Root/ Shoot (DW bases)
	No.	FW	FW	DW	FW	DW	
1	5	151.6	80.9	60.7	25.8	19.3	0.32
2	27	590.8	848.4	635.2	165.2	124.6	0.20
3	6	159.1	105.2	79.81	29.7	21.9	0.27
4	4	93.1	91.3	69.6	23.4	18.5	0.27
5	25	550.6	814.8	615.7	147.7	112.3	0.18
6	11	191.7	177.2	135.6	41.3	30.4	0.22
7	23	489.3	93.6	81.3	18.2	14.4	0.18
8	23	501.7	102.9	90.5	19.8	15.2	0.17
9	19	391.6	78.3	66.7	17.9	13.6	0.20
10	19	289.4	69.8	55.8	16.5	12.8	0.23
11	15	204.1	58.8	46.4	15.9	12.4	0.27
12	14	197.2	49.7	45.9	15.5	11.2	0.24
13	12	185.5	44.8	39.4	14.8	10.2	0.26
14	10	170.3	39.9	33.9	11.5	9.3	0.27
15	10	162.1	34.9	27.7	9.6	8.1	0.29
16	9	159.2	34.2	28.8	8.9	7.3	0.25
17	8	154.9	33.3	26.5	7.8	6.4	0.24
18	7	149.3	30.4	25.6	7.1	5.3	0.21
19	9	159.9	33.9	27.7	7.6	6.5	0.24
20	8	153.6	33.1	26.8	6.9	5.7	0.21
21	7	149.9	32.1	25.4	6.2	5.3	0.21
22	7	144.3	29.3	24.8	5.8	4.7	0.19
LSD (0.05)	6	28.8	19.4	8.7	4.8	9.3	
CV (%)	4	9.7	6.1	2.6	6.1	2.4	

LSD (0.05) for reducing sugars is 22.9 and for glucose is 18.6.
***, refer to (Table 1).**

Regarding vegetative yield, full NPK- amended plants exhibited extraordinary shoot fresh/dry biomass, a finding that not recorded in case of other treatments.

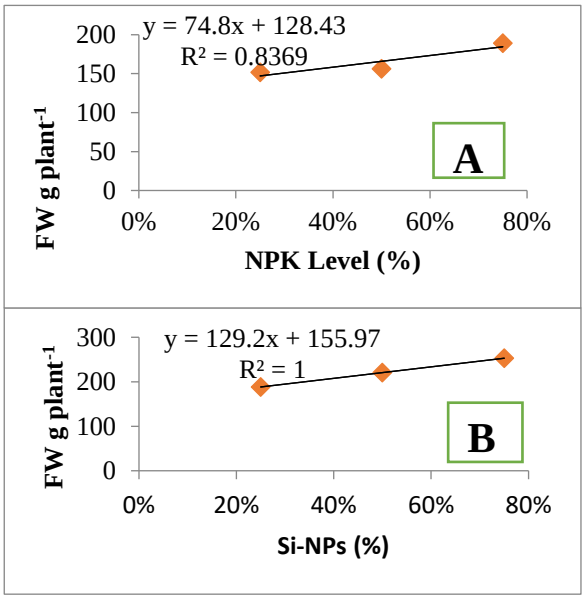

Fig. 5. Linear regressions and coefficient of determination between potato tuber fresh weights and either NPK fertilizer (A) and Si-NP application levels (B).

It is an interesting observation that, although Si-based nanoparticles greatly supported tuber yield, their effects on vegetative growth was not that significant where shoot fresh and dry weights hardly exceeded 105 and 81 g plant^{-1} respectively. In general, fluctuations in either fresh or dry biomass of potato roots among the NPK/Si-NP treatments followed more or less similar trends akin to those recorded with plant shoots. The root/shoot ratio, based on biomass of both, is a particular criterion that indicates the direct impact of a given variable on the root development and consequently the plant growth and crop productivity, the wider the ratio the higher supporting effect. Apart from untreated potatoes, the root/shoot ratios slightly varied among the applied treatments, being falling in the range 0.17-0.29. Even in presence of *F. solani*, simultaneous treatment of 75 or 50 % NPK with any Si-NP level resulted in somewhat wider ratios (0.23-0.24) compared to the fungicide rootolex-treatment (0.18) plants. Hormone profiles of potato shoots and roots obviously varied among the applied treatments (Table 6). Shoots and roots of potatoes simultaneously supplied with the full NPK fertilizer and the fungicide Rootlex scored the highest level of IAA even in presence of *F. solani*, 0.146 and 0.154 ppm were estimated respectively. Silica-nanoparticles, even at the highest application rate, had no supportive influence on the hormone amount in plant organs. Apart from treatment, root system contained 23.3 % more IAA than shoots. As high as 0.125 ppm of ABA, in average, was accumulated in shoots of the full NPK-dressed vegetable plants together with 75 and 100 % of nanoparticles in presence of the pathogenic fungus. Potato roots seemed not proper accumulator for ABA, where they contained *ca.* 47 % of shoot pool. All of all, the level of IAA in plant tissues exceeded that of ABA by 41.4 %.

4. Discussion

It is well established that agriculture is the backbone of the economy particularly in most developing countries. With the rapidly growing day-by-day worldwide population that is predicted to reach *ca*. 8 billion by 2025 and possibly 10 billion by 2050, the global agricultural productivity must be increased to satisfy feeding demands. Besides, the industrial activities and technological applications in recent years are causing harmful effects to human live and the ecosystem. Here, the importance of adopting environmentally friendly strategies for treatment/recycling of the resulting organic and inorganic wastes is of special interest. For the improvement of crop productivity and securing healthy and safe agricultural products, nanotechnology provides new agrochemical materials in addition to new delivery mechanisms [26]. Recent researches in the area of nanotechnology applications proved that this approach can boost agriculture production, which includes the nano-formulations of agrochemicals for using as pesticides and fertilizers for crop proper yields, nano-biosensors for crop protection and nano-devices for genetic manipulation of plants [27, 28, 29, 26]. The beneficial effect of a nanoparticle on plants depends on its size, shape, quantity and application phase, in addition to its biochemical and physical properties. Several types of nanoparticles were reported to communicate directly with plants, altering their morphological features and physiological activities in different ways.

As reported by [30, 26, 31] nanoparticles appeared to act as a strengthening material responsible for supporting disease resistance of a variety of plants *via* restricting/preventing bacterial, fungal and nematode infections. Due to the importance of the silicon element for plant growth, akin to some other essential macronutrients, scientists in the area of

nanotechnology strategies have paid much attention on applying silica nanoparticles for improving crop productivity. Besides, Si deficiency was reported to be linked to nutritional imbalance, resulting in poor growth and yield.

Table 6. Indole acetic acid (IAA) and abscisic acid (ABA) pools ppm of potato shoots and roots of the different experimental treatments.

Treatments*	IAA		ABA	
	Shoots	Roots	Shoots	Roots
1	0.067	0.072	0.053	0.012
2	0.157	0.168	0.143	0.093
3	0.074	0.083	0.062	0.022
4	0.061	0.068	0.051	0.010
5	0.146	0.154	0.102	0.072
6	0.069	0.073	0.058	0.016
7	0.014	0.149	0.137	0.068
8	0.013	0.015	0.132	0.067
9	0.013	0.135	0.123	0.065
10	0.118	0.126	0.114	0.062
11	0.099	0.111	0.041	0.059
12	0.087	0.097	0.085	0.054
13	0.082	0.088	0.079	0.043
14	0.079	0.081	0.076	0.039
15	0.072	0.078	0.073	0.031
16	0.069	0.075	0.064	0.024
17	0.068	0.074	0.063	0.022
18	0.065	0.072	0.059	0.016
19	0.063	0.069	0.057	0.012
20	0.061	0.067	0.055	0.010
21	0.059	0.066	0.054	0.009
22	0.058	0.062	0.049	0.008

***, refer to (Table 1).**

Hence, the idea arose in the present study to throw some light on to what extent silica-based nanoparticles (Si-PNs) might have an antifungal activity towards the pathogen *Fusarium solani*, the causative microbe for root rot disease in potatoes as well as *Aspergillus niger*. Besides, to answer the question….How far these special formulations do contribute to the plant development, productivity and resistance to the pathogen? Actually, this particular bio-foe is known to produce mycotoxins, sambutoxin, trichthecene, fusarin C, fusaric acid mzearalenone and deoxynivalenol [32, 13]. The implemented experimental scenario included: 1) production of Si-PNs from the liquid solution of alkaline-treated sugarcane bagasse, 2) antifungal potential of the produced material towards *F. solani* adopting the agar-disc diffusion technique, 3) the applicability of Si-PNs for supporting both potato growth and tolerance to the pathogen and 4) conversion of cellulose pulp into ß -glucosidase enzyme by *Aspergillus niger*.

A unique pattern of antagonism against *F. solani* was observed in presence of Si-NPs in PDA culture medium. In general, the pathogen growth-inhibition zones measured 0.4-0.9 mm in diameter, an effect that was nanoparticle application dose-dependent. The pathogen did relatively withstand the suppressive influence of Si-NPs of low level, but conspicuously lost a great part of its ability to resist the higher concentrations of the added agent. In this respect, plenty of researches have been dealt with the area of nanotechnological applications for restricting/alleviating the fungal harm and pathogenicity. [33] proved that Si-NPs had potent antifungal activities against a variety of soil fungi that are thought to participate in the regulation of mineral levels. [34] added that these nanoparticles did hamper the growth of the pathogen *Fusarium oxysporum* f. sp. *Niveum* besides reducing the development of the plant parasitic nematode *Meloidogyme incognita*as well as the bacterium *Pectobacterium* and the fungus *Rhizoctonia solani*.

The success of *in vitro* suppression of *F. solani* by Si-NPs in the present study obviously reflected on the behavior of the applied nanosilica regimes towards the pathogen when introduced into soil cultivated with potato. Histologically, the tubers of this particular vegetable plant contains *ca.* 70 % water, a character that makes them vulnerable to galls, blemishes and rots after harvesting during handling, transportation and storage. In the present work, soil infestation of potatoes with *F. solani* severely injured the produced tubers, this is conspicuously expressed in the low yield attributes of the vegetable plant where the number and vigorousness of potato tubers were as low as 4 plant^{-1} of 93.1 g fresh weight. The full recommended NPK fertilization of infected soil did compensate, to a great extent, the deleterious influence of the pathogen resulting in significant increases in number and fresh weight of tubers. Strikingly, the incorporation into soil of Si-NPs particularly at the level of 75 % magnified the vegetable yield, 23 tubers plant^{-1} of 501.7 g were produced. These findings confirmed those of other researchers. [35] mentioned that protection from fungal infection accompanied with enhanced activities of chitinases, peroxidases, PPOs and flavonoids phytoalexins was explored in cucumber as a result of silicon application. With rice and wheat, [36] found that, in the presence of silica both cereals were capable of inducing biologically active substances particularly glycosylated phenolics and phenol content increased during *F. oxysporum* infection. As reported by [37], Si-NP priming of *Triticum aestivum*, *Pisumsativum* and Brassica seeds significantly supported seed

germination and seedling development. In recent studies by [29, 26], nanosilica was successfully used as fertilizers. Additionally, Si-NPs greatly diminished the negative impacts of high salinity on soil relative water content and vegetative growth of Zea mays, resulting in considerable improvement in plant length, fresh and dry biomass, yield and seed quality. They concluded that, maize physiology and anatomy obviously altered following the exposure to nanosilica at 20-40 nm with high surface areas. On the other hand, lower application rates of nanosilica in the present study seemed not beneficial for potato development where the majority of growth parameters decreased compared to untreated plants. This contradicts the results of other investigators [38, 39, 31]. Here, it has to be mentioned that, the discrepancy among scientists on the validity of nanoparticles as bioagents against microbial pathogens indicates that their efficient applicability for better plant development is dependent on their sizes, shapes and application phases as well as physical and biomechanical properties [27].

The possible conversion of cellulose pulp of sugarcane bagasse into reducing sugars and ß -glucosidase enzyme was among the major targets of the present study. Actually, ß -glucosidases a critical constituent of the total cellulolytic complex responsible for catalyzing the final step in cellulose and hemicellulose fractions in the lignocellulosic biomass. It is expressed by all life domains; plants, animals, bacteria, yeasts and fungi. For the industrial applications, microbiota are the pioneer choice for the production of this enzyme, therefore, it received much attention for utilization of cellulose and oligosaccharide substances by bacterial and fungal members for various biotechnological applications. Although high enzyme production rates have been obtained from various carbon sources such as xylan [19], pectin and malt extract [40] and those processes seemed not economic due to the high expenses of the used substances. In the present study, high yield of the of ß -glucosidase was achieved by *A. niger* from cellulose of the low-cost agricultural waste sugarcane bagasse when substituted the carbon source of the recommended synthetic culture medium, where a yield of 16.3 UFP of the enzyme was produced. This is expressed as well, in the high hydrolytic potentials represented by 0.50 and 0.34 mg ml^{-1} reducing sugars and glucose respectively.

Actually, the optimization of fermentation conditions is of special concern for profitable enzyme production and commercialization. Several parameters are needed to be carefully optimized along the fermentation process to magnify the enzyme production. Carbon and nitrogen sources and concentrations, minerals, pH, incubation time and temperature, oxygen availability and inoculum size [41] are the major ones. In the present study, prolonged incubation significantly increased ß -glucosidase production by *A. niger*, up to 500 % increase was estimated with increasing the incubation time from 24 to 120 hr. The rate of increase was significantly low between 96 and 120 hr, a finding might be attributed to the decline in the nutrient availability, accumulation and/ toxicity of by-product or the decrease in the stability of the enzyme itself. In conformity with these findings, [41] reported optimal ß -glucosidase production by *A. niger* and *Trichoderma* sp. after 4 and 5 days of fermentation respectively. [42] mentioned that *Penicillium purpurogemum* and *Chaetomium thermophilum* produced ß -glucosidase properly after 96 and 140 hr., respectively.

As to fungal culture shaking speed, the investigated *A. niger* in the present work, successfully produced the highest ß -glucosidase yield of (114.2 IU/ml) at 200 rpm confirming the results of [43] that increasing aeration rate enhances the dissolved oxygen level in culture medium and this, in turn, supports cell growth and enzyme production. On the contrary, [44] mentioned that, although agitation provides sufficient mixing but could also induce undesirable shear damage to microbial cells and deleterious impacts on enzyme production. However, the appropriate shaking rate would be dependent on the reactor scale and impeller design and should be carefully optimized in reactor scale up.

In the present study, the low-cost corn steep liquor (CSL) was experimented as a supplement to support ß -glucosidase production and hydrolytic potential of the fungal candidate. Raising the quantity of the waste in cultivation medium significantly increased the enzyme production and its hydrolytic potential. The concentration of 3.0 % deemed the most appropriate and resulted in the highest enzyme yield of 72.7 IU/ml accompanied by the highest reducing sugars and glucose contents of 2.21 and 1.50 mg ml^{-1}. In accordance with these findings, [45, 46] reported the advantages of CSL and wheat bran over other raw materials for ß -glucosidase production by *Aspergillus niger*.

To guarantee sustainable ß -glucosidase production via avoiding the expected feedback inhibition of the enzyme, a 12-hr. short term experiment was executed

where distilled water was added to the fermentation medium for periodical removal of the reaction products whenever present. Simply, introduction of 5 ml drop-wise distilled water proved sufficient to alleviate the undesirable effect of the products upon accumulation, an interesting finding that strongly recommended for higher and continuous production rates of the enzyme.

5. Conclusion

The development of an integrated fungal disease management for proper potato productivity that generally encompasses the use of certified disease-free seeds, avoiding the injury of tubers during harvesting, curing and transportation, optimal storage conditions and applying the recommended quantities of registered agro-chemicals could be highly modified by the adoption of nanotechnological strategies. The benefits of silicon to various crops have long been established implying the necessity of Si fertilization of agricultural lands suffering from Si deficiency. In this study, a green route was developed to prepare silica-based nanoparticles from the alkaline-treated sugarcane bagasse waste that authenticated as potential bioagent against *Fusarium solani* responsible for root rot in potatoes. An effect that extended to support the growth and yield of potatoes even when cultivated in soils suffering from fungal diseases. For better understanding the magnitude of silica-based nanoparticle application for satisfying the appropriate and prospective crop productivity, particularly in fungal-diseased environments, the application of the concept under natural field conditions is highly recommended. In addition, upcoming research attempts must focus on securing novel microbiota that possessing high ß -glucosidase production efficiency on low-cost natural raw materials, thermo stability and glucose -tolerance for making biomass hydrolysis cost-effective and profitable.

6. Conflicts of interest

There are no conflicts to declare.

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