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Morphological and Metabolic Response of *Fusarium moniliforme* to Tellurium Stress

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Abstract: *Fusarium moniliforme* was able to grow in the presence of different concentrations of sodium tellurite (0.1-2.0) but at 3.0% (w/v) the fungus morphology was completely distorted. Growth of the fungus was decreased greatly by the increase of metals concentrations. Dark colour colony and black reverse were formed in the presence of tellurite. The fungal biomass was slightly stimulated. The presence of tellurium in the growth medium increased the cellular contents of carbohydrates, proteins and lipids. Moreover, the presence of tellurium induced the biosynthesis of several types of low molecular weight proteins. Total phospholipids were decreased with increasing tellurium concentrations.

Key words: Tellurium, mtalloprotein, *Fusarium moniliforme*

INTRODUCTION

Fusarium moniliforme is one of the most prevalent fungi found in basic human and animal dietary samples. This fungus has been suspected of being involved in human and animal diseases.

In recent years there has been increasing concern about heavy metals toxicity of both aqueous and terrestrial environment. A number of reviews has considered the effects of heavy metals toxicity on fungi[1].

Some metals are essential and required in minute amounts by most living organisms for normal cellular growth, while others are nonessential, fulfill no known cellular role. Although, some heavy metals may be essential for maintaining growth at low concentrations, they are usually toxic at high concentrations.

Metal tolerance in some fungi accumulate heavy metals to concentration that would cause severe toxicity in higher plants[2]. It has been reported that fungi can tolerate heavy metal toxicity either by (1) avoidance, restricts, metal entry into the cells, by reducing the uptake active efflux either by formation of complexes outside the cells[3-5] or (2) sequestration, reduces levels of free ions in the cytosol by the synthesis of organochelators. These organochelators were identified as non enzymatic metalloproteins, metallothionins, short peptides chains (chelators) and very short peptides chains, chelatins[6-11].

The inhibitory action of Tellurium on the growth rate of various fungi was reported e.g. *Cunninghamella echinulata* and *Alternaria tenuissima*[12]; *Aspergillus niger* and *Penicillium chrysogeneum*[13] and *Aspergillus parasiticum* Var. *globosus*[14]. El-Meleigy[15] stated that *Ulocldium atrum*, *Alternaria* and *Aspergillus fumigatus* tolerated high concentration of tellurium. The presence of Te in the culture media caused a distortion in the morphology characteristics of the fungi and increased their cellular contents of proteins and carbohydrates. Interestingly, 20 mg (%, w/v) of Te enhanced *A. alternata* condition. The fungi absorbed considerable quantities of Tellurium. Tolerance indexes of these fungi for Te indicated that fungi tolerated more quantities of Te. Abbass *et al.*[16] reported that *Aspergillus niger* and *Penicillium chrysogenum* were cultured on sulfur-free medium amended with 0.1% (w/v) tellurite. The cell free extract of both strains were fractioned on Sephadex G100 and low mol.wt. Proteins were further fractioned on Sphadex G50. Both fungal strains were found to possess biosynthetic pathways for the biosynthesis of several low and high mol.wt. proteins which serve mainly as tellurium chelators within their cells. Low mol. wt. Proteins were characterized as telluro and non-tellurothioneins. This investigation aimed to study the effect of tellurium on the morphological characters and some metabolic activities of *Fusarium moniliforme*.

MATERIALS AND METHODS

Organism: *Fusarium moniliforme* was obtained from Mycology Center, Assuit University Culture Collection, Department of Botany, Faculty of Science, Assiut University, Egypt.

Growth studies: Aliquots of Czapeks-Dox medium were prepared with different concentrations of sodium tellurite

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0.1, 0.5, 1.0, 2.0 and 3.0% (w/v) . After the poured plates were set, each was inoculated with a 5 mm diameter disc cut from a young active culture of *F. moniliforme* (three replicate plates for each metal concentration). The plates were incubated at 25°C for 7 days, after which the colony diameters were measured.

Replicates of Erlenmyer flasks (250 mL) each containing 50 mL portion of Czapeks-Dox liquid medium amended with the various metal concentrations, were inoculated with 2 mL spore suspension of the organism containing $1x10^6$ conidia mL^{-1}. Control was set without adding Te. The flasks were incubated at 25°C for 10 days statically. Mycelia were harvested by filtration using Bucher funnel. They were then thoroughly washed with distilled water and dried at 60°C for 24 h to constant weight and used as a measure for growth rate.

Morphological features examination: Slides mounts, using Lactophenol blue, were made from control cultures as well as from different metal stressed cultures. All observation and photographs were carried out by a research microscope equipped with a video camera attached to a computer unit.

Preparation of cell free extract: The mycelia were harvested for each Te concentration was homogenized with an approximately equal volume of 70% (v/v) ethanol using a MSE homogenizer. The obtained slurry was centrifuged at 6000 rpm for 10 min, supernatant was collected and concentrated using avacum desiccator.

Protein determination: Protein content was determined quantitatively with Folin-phenol reagent using bovine serum albumin as a stantared protein according to the method of Lowery *et al.*[17].

SDS Polyacrylamide Gel Electrophoresis for proteins: The SDS Polyacrylamide Gel Electrophoresis (PAGE) was carried out in 10% polyacrylamide gel slaps for total soluble proteins of the control cultures and Te treated cultures grown in different Te concentrations according to the method of Laemmli[18]. Protein bands were stained with Coomassi Blue R-250 by standard technique. The protein patterns were analyzed quantitatively using computerized Gel Documentation System (GDS).

Carbohydrate determination: Carbohydrate content was determined quantitatively using the anthrone reagent according to Umbriet *et al.*[19]. Sucrose was used as a standard carbohydrate.

Lipid determination: The total lipid content was determined as described by Bligh and Dyer[20].

Phospholipids determination: Under different treatment with heavy metal, the fungal mycelia were obtained and lyophilized. Three hundred milligrams of lyophilized biomass was extracted with Folch reagent for 18 h and centrifuged for 10 min at 5000 rpm. Then the filtrates were evaporated and re-extracted with Folch reagent, where it gave two layers. The lower layer is the lipids[21]. Lipids were spotted into Silica gel G (Plates) and separated through two dimensional thin layer chromatography[22]. The separated spots were compared with authentic phospholipides (Phosphatidylglycerol, phosphatidyl ethanol amine, phosphatidyl Choline, phospholipids containing Glucosamine, phosphatidyl Insitol momoside).

RESULTS AND DISCUSSION

Growth responses: *Fusarium monliforme* was able to grow in the presence of different tested concentration of Sodium tellurit up to 3.0%. At 0.1% Tellurite containing medium, the radial growth of the fungus was slightly increased (Table 1).

In the presence of 0.5-2.0% tellurite, the radial growth of the fungus was greatly decreased by the increase of the metals concentrations.

On the other hand, the fungus growth was inhibited with 61.1 at 3.0% of Sodium tellurite (Table 1). This result is almost consistent with the previous result. He reported that the radial growth of *A. niger* and *A. tamarii* was decreased by the increase of tellurium concentrations (0.05-0.5%)[23]. Razak *et al.*[12] examined the effect of tellurium at 0.05-3.0% on the growth of *A. niger* and *P. chrysogenum* growing at 28±2°C for 8 days on a Dox agar medium and reported that the radial growth of the two fungi was decreased with increasing tellurium concentration. The colony colour was changed from white in case of control to dark with white nonsporulating margin in the presence of 0.05-2.0% tellurite. The white margin was increased from colour less in control to black in presence of 0.05-2.0%. White precipitation was formed with the black reverse colour at 0.5-3.0% tellurite. Abou-Dahab[23] observed that the fungal colonies of *A. niger*, *A. tamarii, Penicillium citrinum, Cunninghamella echinulata, Paecilomyces variotii* and *Alternaria tenussima* attained black colouration in presence of Potassium tellurite and demonstrated that these colourations were due to the reduction of tellurite and deposition of elemental tellurium within the fungal cells as well as the growth medium. The tested fungus was metabolized tellurium to produce strongly reducing and oxidizing agents into the external environment.

Table 1: The effect of tellurite on growth of *F. monliforme* grown on Czapeks-Dox agar medium at 28°C for 7 days

Na_2TeO_3 concentration% (w/v)	Colony diameter (cm)	Colony colour	Reverse colour	Dry weight (mg mL^{-1})	Inhibition percentage
Control (0.00)	8.0±0.5	White	Colourless	13.4	0.0
0.1	4.9±0.4	Gray with non sporulated margins	Black	9.4	29.8
0.5	4.6±0.5	Gray with non sporulated margins	Black	8.2	38.8
1.0	3.4±0.1	Gray with broad non sporulated margins	Black with white ppt.	8	40.3
2.0	2.3±0.1	Dark gray with broad non sporulated margins	Black with white ppt.	7.3	45.5
3.0	1.5±0.1	Dark black	Black with white ppt.	5.2	61.2

The values of colony diameter are means±standard deviation based on five replicates.

Table 2: Total proteins, carbohydrates, lipids and absorbed Te in the cell free extract of *F. moniliforme* cultivated in Czapeks-Dox medium amended with different Na_2TeO_3 concentrations

Na_2TeO_3 concentrations % (w/v)	Te (ppm)	Proteins (mg g^{-1} dry.wt.)	Carbohydrate (mg g^{+})	Lipid (mg g^{+})	Absorbed Te (%)
Control	0.0	20.5	16.0	3.1	0.0
0.1	$2.9x10^2$	33.0	38.0	4.2	21.0
0.5	$5.8x10^2$	35.0	30.0	10.0	36.0
1.0	$11.6x10^2$	49.7	26.0	14.2	84.09
2.0	$17.4x10^2$	61.7	24.0	12.6	86.0
3.0	$2.9x10^2$	87.7	30.0	5.9	33.0

Tucker *et al.*[24] reported that the precipitate of metallic tellurium in growing cells of *Streptococcus faecalis* and *Corynebacterium diphtheria* as was shown by x-ray diffraction analysis. The metal in the presence of Sodium tellurite was not complexed to any significant degree with any organic material. Ramadan *et al.*[25] noticed a red colouration of the agar medium as well as liquid media when *Fusarium* sp. was cultivated on selenium containing media. They concluded that this colouration was interpreted as a biological reduction of selenium compounds into elemental selenium forming colloidal selenium.

Dry weight responses: The fungus biomass was reduced by 29.0 at 0.1% but at 0.5% Sodium tellurite reduced to 38.8%. Inhibition of the fungus biomass reached 40.3 and 45.5% in presence of 1.0 and 2.0%, respectively, while in presence of 3.0% concentration the reduction was 61.2% (Table 1). Razak *et al.*[13] reported that the reduction in mycelial dry weight of *A. niger* ranged between 2.8-44.5% in presence of 0.05-3.0% potassium tellurite in medium incubated for 8 days at 28±2°C .

Generally, it was observed that all concentrations of tellurite inhibited the fungal growth during the first few days of incubation. This result almost agree with that obtained previously by Zohri *et al.*[14]. They reported that *Aspergillus parasiticus* Var. *globosus* biomass was slightly decreased at lower concentrations and highly inhibited at higher concentrations of tellurite. It was shown that fungal spores were not killed by tellurite but their germination was inhibited and tellurite rapidly reduced and deposited as elemental tellurium within the fungal cells.

Morphological responses: The fungus heavily grown on tellurium free medium giving white cottony like appearance fungal growth with conidia straight and septate (Fig. 1A).

It is quite clear that the fungal hyphae and conidia attained dark colouration at 0.1% tellurite, where the conidia become thicker and shorter, (Fig. 1B). While at 0.5% the fungal conidia septum disappeared and conidia appeared few, small and plasmolysed (Fig. 1C). At 1.0% fungal hyphae appeared enlarged and plasmolysed with no conidia (Fig. 1D). At 2.0% fungal hyphae appeared completely plasmolysed (Fig. 1E). Slight growth was detected at 3.0% and the fungal hyphae were completely distorted (Fig. 1F). The intensity of the dark coloration of the fungal hyphae was increased with increasing sodium tellurite concentrations in the medium. This is probably a result of the deposition of elemental tellurium within the fungal cells causing inhibition of the vital processes. Interestingly, the fungus excreted elemental tellurium on the outer surface of the mycelia.

Our data are almost consistent with Razak *et al.*[6], El Meliegy[15] and Zohri *et al.*[14] where the presence of tellurite in the growing media caused morphological distortions of several fungi.

Effect of tellurium on fungal proteins, carbohydrates and lipids: Increasing ratio of tellurium from 37.0% in case of 0.1% Te to 75.6% in 3% Te (Table 2) increased the total fungal soluble protein. The maximum quantity of total proteins obtained at $2.9x10^3$ ppm Te and the maximum quantity of total carbohydrates obtained at $2.9x10^2$ ppm Te, while the maximum lipids quantity was at $11.6x10^2$ ppm Te. However the maximum quantity of absorbed Te was achieved at $17.4x10^2$ ppm. The fungus altered the metabolic activities to overcome the presence of high Te concentration in the environment or loss its ability to regulate the permeability of ions into the cells. Ramadan *et al.*[26] stated that *Aspergillus fumigatus*,

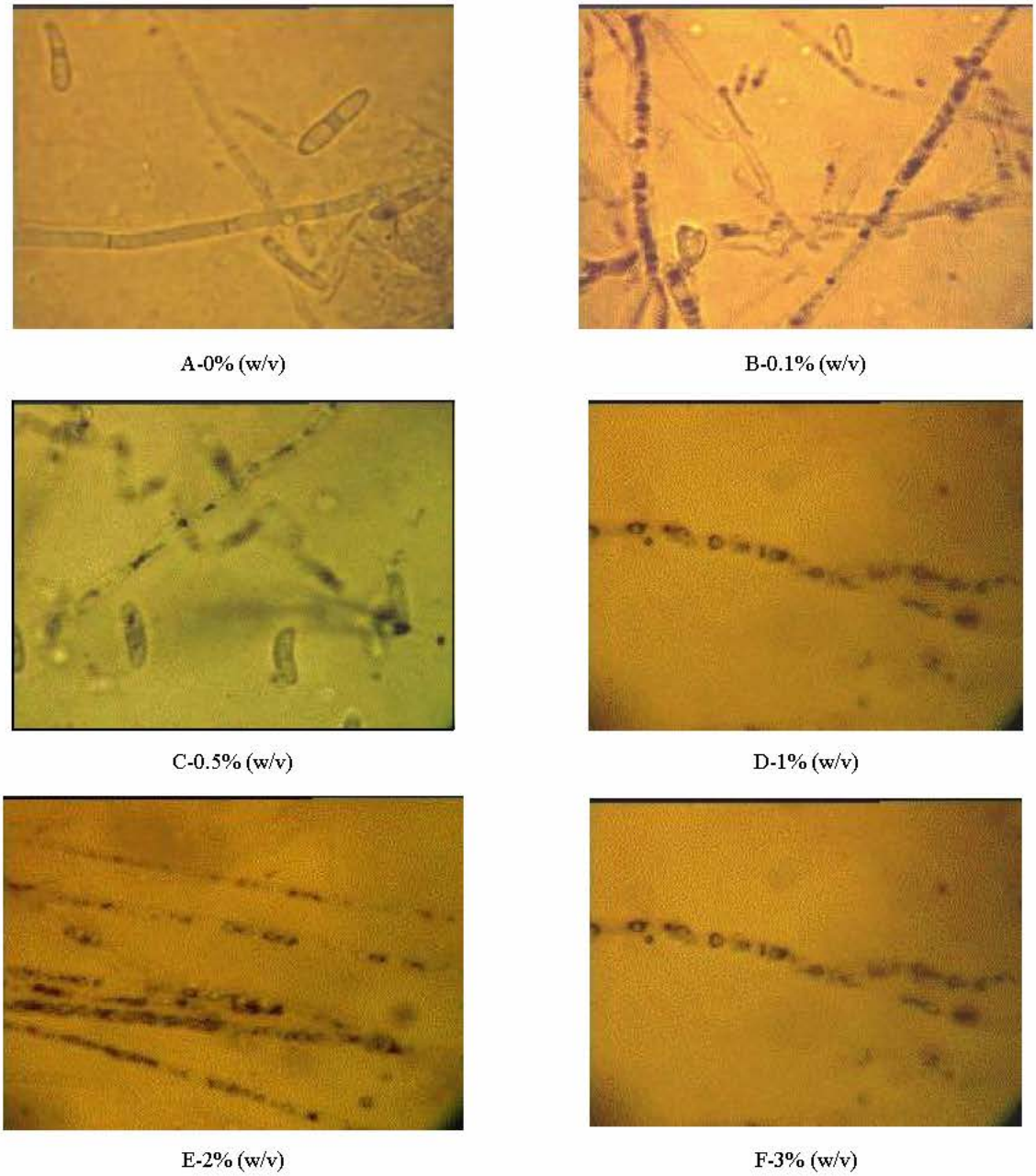

Fig. 1: Effect of Te on *Fusarium moniliforme* morphology. The fungus grows well on medium lacking tellurium giving white cottony like appearance fungus growth with straight conidia and septated hyphae (A). The hyphae and conidia attained dark colouration at 0.1% tellurite, where the conidia become thicker (B). While at 0.5% the fungal conidia septum disappeared and conidia appeared few, small and plasmolysed (C). The fungal hyphae appeared enlarged and plasmolysed with no conidia at 1.0% (D). At 2.0% fungal hyphae appeared completely plasmolysed (E). Slight growth was detected at 3.0% and the fungal hyphae were completely distorted (F). Magnification was x 400.

A. terreus and *Penicillium chrysogenum*, are Te-tolerant fungi and grew on S-free medium amended with 0.2% Na_2TeO_3. They have referred the fungi tolerance to Te into its incorporation to several types of low and high mol.wt. proteins. The newly detected telluroproteins contained an extra-ordinary high level of Te, as well as tellurocysteine, tellurocystine, telluromethionine and serine.

Table 2 indicates higher protein levels, carbohydrates and lipids in the fungal extracts cultured on tellurium containing media other than that of control. This increase in these metabolic product might be because of osmotic equilibration as well as an activation of the biosynthesis of several adsorbents within the fungal cells.

Absorption of tellurium by *F. moniliforme*: Table 2 indicate the relative high absorption of tellurium by the fungus. Maximum concentration of tellurium absorption were detected at 1 and 2% Na_2TeO_3. Unexpectedly, a lower absorption of tellurium was detected at 3%. These results indicate irregular pattern of tellurium absorption as a function of tellurium concentration in the growth media. Apparently, the absorbed tellurium is not dependent on the tellurium content in the growth environment. More likely, it depends on factors that influence tellurium absorption, such as cell permeability and several other factors.

Different Te concentrations induced some variation in the banding patterns of proteins extracted either by Na-phosphate or Tris-HCl buffer. These variations are revealed by alterations in banding intensity, and the percentage of protein in each band compared to the total amount, number of peaks in scanning profiles (Table 3 and 4). The presence of some new polypeptides by some treatments using Tris-HCL buffer is demonstrated by the presence of new bands with M.wt. >104, 128 and 135 kDa and in Na-phosphate buffer by the presence of new bands with M.wt. >98, 77, 32 and 29 kDa. The disappearance of polypeptide by some metal treatments may be demonstrated by bands with M.wt 19 kDa when using Tris-HCL buffer and the absence of bands with M.wt. 16 kDa when using Na-phosphate buffer.

The over-expression of some bands is demonstrated by bands with M.wt. 20 kDa in Tris-HCL and 56, 19, 15, 14 and 13.5 kDa in Na-phosphate. This change may be attributed to alteration in structural genes or to changes in the expression of regulatory genes involved in regulating the concerned structural genes. The occurrence of additional bands in PAGE profile may be the result of synthesis new proteins controlled by a structural gene to overcome the effect of metal concentrations.

Table 3: Relative concentration of proteins extracted with Na-phosphate buffer, pH 7 from *F. moniliforme* identified by the SDS-PAGE profile and data were analyzed by the computerized Gel Documentation System

M.wt. (kDa)	Control	0.1% Na_2TeO_3	0.5% Na_2TeO_3	1.0% Na_2TeO_3	2.0% Na_2eO_3	Remarks
99	--	0.4	--	--	--	1
98	1.9	1.9	2.9	2.7	2.6	1
96	1.7	2.7	1.7	1.7	2.6	1
78	1.3	1.7	2.5	0.7	2.1	1
77	--	--	0.9	--	--	1
76	2.9	1.9	1.6	1.2	3.1	1
56	14.8	19.0	19.9	20.4	21.1	1
54	5.8	--	1.0	--	0.9	2
50	1.3	0.4	0.3	--	--	2
49	0.5	0.2	1.3	0.3	0.7	1
43	--	--	1.5	--	--	1
36	1.8	0.4	6.7	3.2	5.1	1
34	10.4	4.5	6	6.1	6.6	1
32	--	2.5	--	--	--	1
29	--	5.9	--	--	--	1
25	16.0	15.9	9.7	15.1	15.0	1
22	3.4	--	--	2.9	2.7	2
20	7.0	--	17.1	15.0	21.0	1
19	9.2	24.5	--	16.7	18.2	1
17	--	--	13.0	17.1	15.5	1
16	4.9	--	--	4.4	--	1
15	3.9	3.4	5.5	5.1	4.3	1
14	0.2	6.3	9.6	7.7	5.8	1
13.5	3.6	5.0	4.0	4.8	3.9	1

1 Band induced or increased with Na_2TeO_3 treatment
2 Band decreased or disappeared with Na_2TeO_3 treatment.

Table 4: Relative concentration of proteins extracted with Tris/HCl, pH 8.2 buffer from *F.moniliforme* identified by the SDS-PAGE profile and Data were analyzed by the computerized Gel Documentation System

M. wt. (kDa)	Control	0.1% $Na_2Te\ O_3$	0.5% $Na_2Te\ O_3$	1.0% Na_2TeO_3	2.0% $Na_2Te\ O_3$	Remarks
128	--	--	--	0.3	0.8	1
104	9.9	3.1	2.4	9.6	4.0	1
99	9.3	6.0	--	1.4	2.1	2
88	--	--	--	--	1.8	1
48	21.6	25.3	20.2	22.9	2.2	1
46	3.2	6.4	4.3	--	3.7	2
39	2.4	--	6.7	6.9	2.2	2
29	21.6	20.7	22.3	23.3	21.2	1
28	--	3.4	--	--	--	1
27	6.4	5.9	6.9	7.5	7.5	1
20	11.4	19.0	14.8	15.9	16.1	1
19	3.9	--	--	3.1	38	2
18	5.4	5.8	9.0	6.7	6.5	1
16.5	1.8	0.9	1.3	0.6	1.0	1
15	0.8	1.7	0.8	0.8	1.0	1
14	1.4	0.8	1.8	1.4	3.0	1
13.5	--		--	1.1	--	1

1 band induced or increased with Na_2TeO_3 treatment
2 band decreased or disappeared with Na_2TeO_3 treatment

Effect of tellurium on phospholipids: Total phospholipid decrease with increase tellurite concentrations (Table 5).

Phosphatidylglecrol showed the greatest increase in cell membranes with increased tellurium concentration in the media (Table 6). While Phosphatidyl Choline has the same concentration in all tellurium concentration in comparing with control.

Table 5: Effect of tellurium on phospholipids of *Fusarium moniliforme*

Na_2Te0_3 concentrations % (w/v)	Control	0.1	0.5	1.0
Total phospholipids (mg/gm dry wt.)	0.173	0.150	0.080	0.052

Table 6: Composition of phospholipid types from *Fusarium moniliforme* fungal isolates grown at different concentration of tellurite

Phospholipid types	Control	0.1	0.5	1.0
Phosphatidyl glycerol	+	+	++	++
Phosphatidyl ethanol amine	-	-	-	-
Phosphatidyl Choline	+	+	+	+
Phospholipids containing glucosamine	-	-	-	-
Phosphatidyl instol monoside	+	-	-	-

- = Fungus contains nothing, + = Fungus contains small amount
++ = fungus contains high amount

Phosphatidylethanolamine and Phospholipids containing Glucosamine showed absence in cell membranes of the tested organism under Te different tested concentrations.

Hefnawy[27] reported that total possible explanation was given as a result of precipitation of elemental tellurium within the cells, whereas the appearance of black coloration in the external medium is attributed to the precipitation of elemental tellurium in the medium through the action of strong estimated quantities of total proteins, carbohydrates and lipids in the fungal cells cultivated on tellurium containing media were markedly increased than those grown on tellurium free media. This was explained as a mode of osmotic activities to minimize the external osmotic stress resulting from the presence of tellurium as well as synthesizing Te-binding compounds and more likely disturbance in the metabolic activities leading to the accumulation of such compounds.

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