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EFFECTS OF MERCURY RESISTANT BACTERIA ON THE GROWTH OF *TRITICUM AESTIVUM* L. SEEDLINGS AT DIFFERENT CONCENTRATIONS OF MERCURIC CHLORIDE

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Key words: Murcuric chloride, Hg-resistant bacteria, *Triticum aestivum*, Waste water

Abstract

Fifteen mercury resistant bacteria (*viz*., AzHg-1 to AzHg-15) were isolated from sewerage and industrial effluents from Kasur, Pakistan. These isolates could resist 200 µg/ml of $HgCl_2$ in the medium. The ability to promote early growth and their effect on metal uptake by a variety of *Triticum aestivum* i.e., Pak-81, was screened out at different concentrations (0, 1, 2 and 3 mM) of mercury. Different growth parameters (seed germination, seedling root and shoot length, seedling fresh and dry weight, dry weight per gram fresh weight) and accumulation of mercury by inoculated and non-inoculated seedlings were determined and recorded. Results demonstrated that mercury adversely affected and reduced the seed germination and seedling growth of *Triticum aestivum*, while Hg content of seedlings increased with increasing concentrations of mercury salt. Almost all bacterial strains had stimulatory effect on the germination and various growth parameters of wheat seedlings under salt stress conditions, when compared to respective non-inoculated treatments, except some isolated cases. Mercury content of seedlings was also decreased in inoculated seedlings.

Introduction

Industrial wastes laden with various chemicals and heavy metals are posing serious problems in Pakistan where the environmental awareness is abysmally low, waste recycling, treatment and disposal of effluents is not according to world standard (Khalil *et al*. 1991). Heavy metals are used in many industries because of their technological importance. The major source of mercury pollution, a non-essential heavy metal, are mining, smelting, chloralkali plants, paper pulp industries, coal burning, natural gas and refining of petroleum products (Basit 1992). With the rapid phase of industrialization and modernization of agriculture, the use of mercury salts and their diffusion into biosphere have become almost unavoidable. Waste water from the industries contaminated with heavy metals cause toxic effects on human beings, plants, animals and environment (Anon. 2004). Heavy metals which are essential in low concentration cause pollution and toxic effects on biota in high concentrations (Klerk and Weiss 1987). Das *et al.* (2013) studied the phytotoxicity of two heavy metals, mercury and cadmium, on an aquatic plant Hydrilla and reported the decrease in chlorophyll content of the test plant. In another study conducted by Libert and Barkay (1988), it was found that mercury inhibits cell elongation in plants. Besides plants mercury also effects bacterial growth. Mercury ions inhibit the biosynthesis and enzymatic activity of bacteria due to binding of sulfahydral group while bacteria exhibit a number of mechanisms for mercury detoxification (Foster 1983, Babich *et al*. 1991). Use of bacterial strains to counter the toxic effects of heavy metals on the growth of plants has been reported by several workers (Burd *et al*. 2000, Abou-Shanab*et al*. 2006).

Present research work was carried out to evaluate the effects of mercury resistant bacteria on the growth of a variety of wheat seedlings exposed to different concentrations of mercury.

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Materials and Methods

Fifteen mercury resistant bacteria (*viz.*, AzHg-1 to AzHg-15) were isolated from sewerage and industrial effluents. These isolates could resist 200 µg/ml of $HgCl_2$ in the growth medium (Fulthorpe *et al.* 1992), while wheat seeds (*Triticum aestivum*, var. Pak-81) were obtained from Punjab Seed Corporation, Sahiwal. Experiment was designed following the methods of Hasnain *et al.* (1993). From experimental growth of the different bacterial strains at 37ºC, 10 µl of inoculum was given in fresh L. Broth which was grown for 16 hrs at the same temperature. After which bacterial strains were collected by centrifugation at 5,000 rpm for 5 minutes. Cells were washed and resuspended in enough sterile glass distilled water so that bacterial population was adjusted to 10^7 cell/ml. Twenty five inoculated and control (non-inoculated) seeds were spread in pre-labeled Petri plates having filter papers. Ten ml of respective mercury solution of different concentrations (0, 1, 2 and 3 mM) was added in their respective plates. All the Petri plates were placed in dark at 25 ± 2ºC for germination. Germination process was recorded daily up to 3rd days. On 3rd day of germination, seedlings were supplied with ten ml of Hewitt's nutrient solution (Hewitt 1963) containing 0, 1, 2 and 3 mM of $HgCl_2$. Petri plates were shifted to 10 K lux light (16 hrs day length) 25 ± 2ºC in the growth chamber. Seedlings were grown for 10 days. After 10 days seedlings were harvested and various growth parameters were studied including seed germination, percentage seedling root and shoot length (cm), seedling fresh and dry weight (g), dry weight per gram fresh weight (mg/g).

To analyze mercury content in the seedlings, wet digestion of the plant material was done in CEM microwave oven according to EPA protocol 3052 for microwave digestion of organic material. Digested plant material was filtered and diluted to get the absorption (Matusiewicz 1997) on the atomic absorption spectrophotometer (spectra AA20). All data were analyzed statistically.

Results and Discussion

Though mercury is non-biodegradable but bacteria exhibit a number of mechanisms for mercury detoxification (Siciliano *et al.* 2002). As microorganisms particularly bacteria have various interactions with other life forms especially plants so they may be helpful in reducing the adverse effects of heavy metals. In present study the effects of mercury and various mercury tolerant bacteria were observed on a wheat variety (Pak-81), results showed that there was a regular decrease in the germination percentage of non-inoculated seeds as compared to inoculated seeds with the increase in mercury concentration (with a few exceptions).

Different bacterial inoculations had different effects on various concentrations. AzHg-4, AzHg-5, AzHg-6, AzHg-10 stimulated germination under all $HgCl_2$ concentrations (significant in many cases), when compared with respective non-inoculated treatments. In addition to these AzHg-1 (1, 3 mM), AzHg-2, AzHg-3 (1, 2 mM), AzHg-7, AzHg-8 (2 mM), AzHg-11, AzHg-13 (2, 3 mM). AzHg-12 and AzHg-15 (1 mM) also caused an increase in percentage germination, over respective non inoculated treatments. Only AzHg-9 caused reduction in the percentage germination at all treatments (Table 1). These results are in accordance to the work of many researchers who have reported the inhibition in germination and reduction in plant growth due to heavy metal toxicity (Iqbal and Shazia 2004, Sharma and Durbey 2005, Meryem and Yasmin 2013), while increase in germination percentage due to bacterial inoculation under various heavy metal stress conditions is also reported (Burd *et al.*2000, Gupta *et al.* 2006).

When seedling length (root and shoot lengths together) is considered, majority of strains caused stimulation at 1 (excluding AzHg-7, AzHg-8, AzHg-11) and 2 mM (except for AzHg-2) treatments when compared with respective non- inoculated treatments. At 3 mM treatments only AzHg-10 and AzHg-11 promoted seedling growth over non-inoculated 3 mM treatment

(Table 2). With increase in mercury concentration decrease in length parameters which may be due to the mercury ion incorporation into the cell wall components or reduction in mitotic activity (Tomer *et al.*2000). Gupta *et al.* (2006) reported reduction in all growth parameters of *Vigna mungo* when treated with different concentrations of lead. Inoculations with bacteria in most of the cases improved germination, seedling length and weight parameters (Kang *et al.* 2012). The bacterial strains caused a non-significant increase only under 3 mM mercury stress.

Table 1. Effects of mercury resistant bacteria on the germination (%) of wheat seedlings under mercury stress (mM).

Isolates	$HgCl_2$ concentrations			
	0 mM	1 mM	2 mM	3 mM
Control	100.000 ± 00.000	9.000 ± 4.714	51.666 ± 5.138	31.666 ± 3.6000
AzHg-1	98.333 ± 1.360	98.33 ± 1.360	45.000 ± 4.240	35.000 ± 1.801
AzHg-2	100.00 ± 0.000	93.333 ± 3.6000	56.666 ± 3.923	23.333 ± 2.525
AzHg-3	96.666 ± 1.360	91.666 ± 2.721	65.000 ± 6.329	30.000 ± 2.236
AzHg-4	100.000 ± 0.000	91.666 ± 4.906	75.000 ± 4.247	75.000 ± 3.071
AzHg-5	100.000 ± 0.000	91.666 ± 1.360	91.666 ± 1.360	53.333 ± 3.810
AzHg-6	100.000 ± 0.000	91.666 ± 6.804	66.666 ± 5.153	67.500 ± 1.767
AzHg-7	100.000 ± 0.000	86.666 ± 4.2000	55.000 ± 6.236	17.500 ± 1.767
AzHg-8	98.333 ± 1.360	88.333 ± 3.600	56.666 ± 4.136	18.333 ± 2.200
AzHg-9	96.666 ± 1.360	88.333 ± 5.443	45.000 ± 4.785	11.666 ± 2.721
AzHg-10	98.333 ± 1.360	100.000 ± 0.000	63.333 ± 8.767	42.500 ± 4.838
AzHg-11	98.333 ± 1.360	85.000 ± 6.274	63.333 ± 5.931	43.333 ± 4.401
AzHg-12	98.333 ± 1.360	93.333 ± 1.360	45.000 ± 0.060	15.000 ± 2.357
AzHg-13	98.333 ± 1.360	90.000 ± 2.357	76.666 ± 6.804	35.000 ± 1.801
AzHg-14	96.666 ± 1.360	86.666 ± 6.804	60.000 ± 6.236	25.000 ± 2.374
AzHg-15	100.000 ± 0.000	91.666 ± 3.600	45.000 ± 6.329	21.666 ± 1.626
LSD (p = 0.05)			14.486	

As for as the weight parameters are concerned, fresh weight of seedlings decreased significantly with the application of $HgCl_2$ treatments while dry weight of seedling decreased at 1 mM but at 2 and 3 mM some increase in dry weight of seedlings, when compared with 0 mM treatment was observed. Bacterial inoculations caused both increase and decreases in this parameter but there was no specific strain based pattern or concentration based pattern. Dry weight per gram fresh weight increased significantly with $HgCl_2$ stresses and there was progressive increase with the increase in concentration of mercury salt. At 0 mM bacterial inoculation generally caused an increase (except AzHg-5) in the dry weight accumulation. But under stress conditions decrease in dry weight accumulation were observed at 1 (except AzHg-1, AzHg-3), 2 (except for AzHg-1, AzHg-2, AzHg-8) and 3 mM (AzHg-1, AzHg-2, AzHg-6, AzHg-7, AzHg-9, AzHg-12, AzHg-14) treatments when compared with non-inoculated respective treatments, with different bacterial inoculations. However, some increase over respective non-inoculated treatments was also observed (Table 3).

Mercury accumulation was determined by analysis of digested plant material through atomic absorption spectrophotometer. Hg-content of seedlings increased significantly with the increase in concentration of mercuric salt in the medium. At 0 mM treatment no Hg-content was detected in seedlings. The increase/decrease in mercury accumulation was neither regular nor specific with

Table 2. Effects of mercury resistant bacteria on the seedling lengths (cm) of wheat under mercury stress (mM).

Isolates	$HgCl_2$ Concentrations			
	0 mM	1 mM	2 mM	3 mM
Control	29.682 ± 1.144	8.726 ± 0.886	4.022 ± 0.494	3.508 ± 0.279
AzHg-1	26.994 ± 1.295	9.031 ± 0.704	4.027 ± 0.322	3.291 ± 0.202
AzHg-2	28.807 ± 1.090	9.264 ± 0.676	2.947 ± 0.609	2.900 ± 0.000
AzHg-3	26.814 ± 1.232	9.047 ± 0.714	5.593 ± 0.406	2.704 ± 0.196
AzHg-4	28.025 ± 1.088	9.582 ± 0.623	5.322 ± 0.504	2.200 ± 0.212
AzHg-5	26.907 ± 1.481	10.002 ± 0.717	6.435 ± 0.407	3.294 ± 0.315
AzHg-6	29.968 ± 1.119	10.028 ± 0.731	4.869 ± 0.399	2.342 ± 0.165
AzHg-7	26.583 ± 1.286	8.647 ± 0.675	4.560 ± 0.386	2.966 ± 0.191
AzHg-8	27.585 ± 1.196	8.513 ± 0.832	4.850 ± 0.369	3.220 ± 0.229
AzHg-9	26.310 ± 1.460	9.136 ± 2.090	5.970 ± 0.268	2.500 ± 0.182
AzHg-10	27.940 ± 1.192	10.368 ± 0.733	4.563 ± 0.414	5.000 ± 0.457
AzHg-11	27.569 ± 1.472	8.437 ± 0.722	4.761 ± 0.412	3.799 ± 0.235
AzHg-12	29.874 ± 1.902	9.669 ± 0.572	4.611 ± 0.337	2.750 ± 0. 147
AzHg-13	29.233 ± 1.373	9.678 ± 0.602	4.245 ± 0.310	2.549 ± 0.179
AzHg-14	29.402 ± 1.345	9.432 ± 0.718	5.572 ± 0.489	2.993 ± 0.333
AzHg-15	28.850 ± 1.185	8.816 ± 0.708	5.787 ± 0.401	3.085 ± 0.192
LSD (p = 0.05)	7.690			

Table 3. Effects of mercury resistant bacteria on dry weight per gram fresh weight (mg/mg) of wheat seedlings under mercury stress (mM).

Isolates	$HgCl_2$ Concentrations			
	0 mM	1 mM	2 mM	3 mM
Control	0.116	0.295	0.376	0.439
AzHg-1	0.119	0.314	0.445	0.500
AzHg-2	0.130	0.280	0.409	0.571
AzHg-3	0.155	0.306	0.341	0.500
AzHg-4	0.129	0.290	0.297	0.400
AzHg-5	0.116	0.260	0.291	0.357
AzHg-6	0.137	0.239	0.295	0.446
AzHg-7	0.118	0.247	0.371	0.492
AzHg-8	0.119	0.264	0.395	0.394
AzHg-9	0.150	0.230	0.302	0.480
AzHg-10	0.130	0.250	0.359	0.326
AzHg-11	0.126	0.272	0.322	0.321
AzHg-12	0.117	0.214	0.282	0.461
AzHg-13	0.120	0.240	0.367	0.362
AzHg-14	0.127	0.229	0.319	0.453
AzHg-15	0.137	0.247	0.318	0.343

reference to bacterial strains or mercury treatments. For instance with bacterial inoculation, generally, Hg-content of seedling decreased at 1 and 2 mM treatments, when compared with non-inoculated respective treatments. At 3 mM, inoculation with AzHg-3, AzHg-11, AzHg-12, AzHg-13 and AzHg-14 caused some increase, over respective non-inoculated treatment, in the Hg-content of seedling. While with rest of inoculations (AzHg-1, AzHg-2, AzHg-4, AzHg-5, AzHg-6, AzHg-7, AzHg-8, AzHg-9, AzHg-10, AzHg-15) decreases in the uptake of mercury content by seedlings was observed (Table 4).

Table 4. Effects of mercury resistant bacteria on the mercury accumulation (mg/g) dry weight of wheat seedlings under mercury stress (mM).

Isolates	$HgCl_2$ concentrations			
	0 mM	1 mM	2 mM	3 mM
Control	0	12.87	19.40	23.87
AzHg-1	0	05.26	09.09	19.76
AzHg-2	0	03.02	07.35	21.77
AzHg-3	0	04.65	17.27	26.31
AzHg-4	0	04.97	07.63	18.13
AzHg-5	0	02.97	04.18	8.98
AzHg-6	0	02.96	03.88	15.88
AzHg-7	0	00.01	10.42	20.25
AzHg-8	0	06.60	06.86	10.03
AzHg-9	0	06.38	09.40	21.25
AzHg-10	0	04.00	04.72	17.78
AzHg-11	0	03.90	07.95	24.20
AzHg-12	0	04.73	07.25	32.73
AzHg-13	0	04.42	04.90	26.36
AzHg-14	0	05.93	07.09	27.63
AzHg-15	0	03.74	04.80	14.55

Bacteria improve the plant growth by increasing the availability of nutrients or resistance against harmful metals or by reducing the availability of metal to the roots (Li *et al*. 2007, Rajkumar and Freitas 2008). Differences in the accumulation tendency of bacterial inoculations might be due to the genetic variability found among them.

Present study showed that mercury tolerant bacteria may be successfully used to improve the growth of wheat under mercury stress. This study also revealed effective plant-microbe interaction is dependent upon genetic variability of bacterial strain.

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