EFFECT OF FUNGICIDE ($CUSO_4$) ON GROWTH AND MORPHOLOGY OF *NEUROSPORA*

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Abstract: Copper is an essential mineral nutrient which is required in trace amount for normal growth and development of living organisms. However, at high concentrations, copper is detrimental to all types of cells. Copper shows high antimicrobial activity towards large number of microorganisms and copper compounds such as copper sulphate, cupric hydroxide and Bordeaux mixture are used as effective biocides. On the other hand, various microorganisms including fungi can grow and adapt to high copper concentration and are being used for bioremediation of copper. Thus, it is of interest to study the effect of different concentrations of copper on growth and morphology of fungi which can grow at high copper concentrations. In the present study we describe the effect of different concentrations of copper sulphate on the growth and morphology of wild-type and mutant strains of *Neurospora* which can grow at high copper concentrations.

Keywords: Bioremediation, copper sulphate, growth, morphology, *Neurospora*.

Introduction: Copper is a heavy metal which is crucial for all living organisms. It acts as a cofactor for various enzymatic processes and is required in small amounts. Excess of this element can be harmful for most living organisms (Saidi, 2010; Dhokpande and Kaware, 2013; Gupta and Kumar, 2012). Copper accumulates in high concentration in environment mostly due to use of fungicide sprays, application of fertilizers, purification of metals such as smelting of copper, preparation of nuclear fuels or from dumping of agricultural and municipal wastes (Gupta and Kumar, 2012; Samal and Kotiyal, 2013). Thus, the contamination of soil due to these anthropogenic activities results in elevated levels of this metal which is potentially toxic to living forms (Gupta and Kumar, 2012). The presence of high concentrations of copper in the environment promotes the selection of microorganisms which possess mechanisms for tolerating high amount of copper and develop resistance mechanisms (Festa and Thiele, 2012). Many microorganisms have been reported which can tolerate high amount of copper (Samal and Kotiyal, 2013; Iskandar *et al*., 2011; Vyas *et al*., 2007). It is important to understand the mechanisms which allow microorganisms to grow and adapt at high copper concentrations (Gupta and Kumar, 2012). Issazadeh *et al.* (2013) and Cervantes and Corona (1994) discussed various mechanisms for tolerating high copper concentration in microbes, like compartmentalization, efflux of metal ions outside the cell, intracellular metal ion accumulation, trapping of metal in cell wall, altered uptake, extracellular chelation by secreted metabolites and intracellular complexing by metallothioneins and phytochelatins. Many microbes which have the ability to uptake high amount of copper from environment are being used for bioremediation of copper (Iskandar *et al*., 2011; Gupta and Kumar, 2012; Kumar *et al*., 2011). In this paper, we have studied the effect of different concentrations of copper sulphate on growth and morphology of different wild-type and mutant strains of *Neurospora*.

Materials and methods:

Isolation and Identification of Neurospora: *Neurospora* conidia were collected on sterilized filter paper from colonies growing on discarded corncobs at railway track of Ujjain (India) (Mukati *et al*., 2012). The filter paper was placed on Vogel`s medium plate containing chloramphenicol and *Neurospora* cultures were isolated (Perkins and Turner, 1988). The mating-type of the cultures was determined using the method of Perkins *et al*. (1989). The cultures were purified and their species were identified by following the method described by Perkins and Turner (1988).

Growth and Morphology: *Neurospora* cultures were grown in Petri dishes containing Vogel's minimal agar medium with glucose as carbon source and having different concentrations of copper sulphate (Davis and de Serres, 1970). The cultures were incubated at 34± 2°C and details of their hyphal morphologies were examined under microscope. The growth rate was determined in race tubes containing different concentrations of copper (Ryan *et al*., 1943).

Mutagenesis of *Neurospora* cultures: The macroconidia of *Neurospora* culture showing best growth at high (400 ppm) concentration of copper were mutagenized using Ethyl Methane Sulphonate (EMS) and mutant strains with ability of tolerating 400 ppm copper were isolated (Davis, 2000) and their growth and morphological features were examined as described above.

Results and discussion: Ten cultures of *Neurospora* were isolated from visible colonies growing on corncobs. The cultures were purified and their mating types and species were determined. The results are shown in table 1. All the cultures produced bright yellow coloured conidia and thus belong to yellow ecotype of *N. intermedia*. The cultures were grown in successively increasing concentrations of copper (0.1- 400 ppm Cu). The wild-type cultures could barely grow directly on 100 ppm copper concentration but when they were grown in gradually increasing concentrations of copper, then they slowly adapted themselves and all the cultures could grow on 100 ppm copper concentration. There were variations in growth rates of the cultures which shows that different strains have different adaptability for copper (Figure 1). These cultures were further grown on 200, 300 and 400 ppm concentrations of copper and the results show that all the cultures could grow on 200 ppm Cu, seven cultures could grow on 300 ppm copper and only three cultures could grow on 400 ppm copper concentration. Thus, there is strain to strain variability and only 30% cultures could grow and adapt at 400 ppm copper concentration. The cultures U8-1, U8-7 and U8-8 showed best growth at 400 ppm copper.

Table 1: *Neurospora* cultures isolated from nature

S. No.	Culture No.	Place of sampling	Mating type	Color of Conidia	Species
1.	U8-1	Railway track, Ujjain	a	Yellow	*N. intermedia*
2.	U8-2	Railway track, Ujjain	a	Yellow	*N. intermedia*
3.	U8-3	Railway track, Ujjain	a	Yellow	*N. intermedia*
4.	U8-4	Railway track, Ujjain	a	Yellow	*N. intermedia*
5.	U8-5	Railway track, Ujjain	a	Yellow	*N. intermedia*
6.	U8-6	Railway track, Ujjain	a	Yellow	*N. intermedia*
7.	U8-7	Railway track, Ujjain	A	Yellow	*N. intermedia*
8.	U8-8	Railway track, Ujjain	a	Yellow	*N. intermedia*
9.	U8-9	Railway track, Ujjain	a	Yellow	*N. intermedia*
10.	U8-10	Railway track, Ujjain	A	Yellow	*N. intermedia*

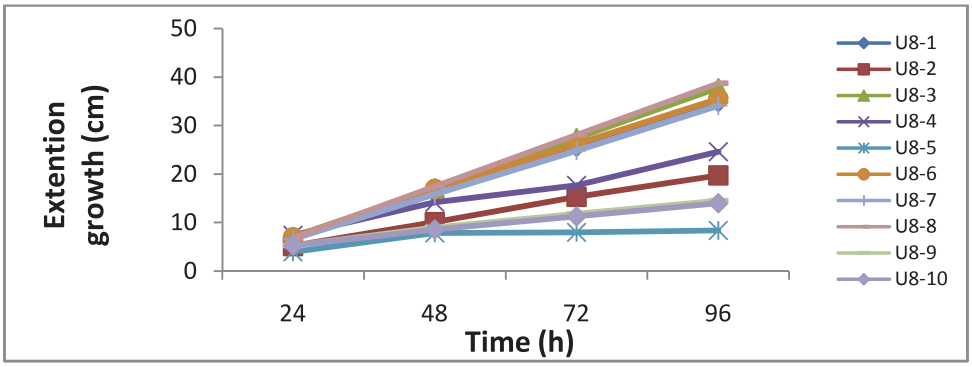

Figure 1: Extension growth of *Neurospora* cultures at 100 ppm Cu.

The macroconidia of U8-8 were mutagenized with EMS to select strains which have better ability to tolerate copper. Five best growing colonies from mutagenized macroconidia of U8-8 which could grow at 400 ppm copper were isolated and were designated as AG-64, AG-66, AG-67, AG-71 and AG-74. The extension growth of these mutants and adapted culture (U8-8 A, where 'A' represents adapted culture) at 400 ppm concentration of copper was determined and is shown in Figure 2. The wild-type culture does not grow at 400 ppm copper. It is clear from the graph that there is strain to strain variability in growth rate at 400 ppm copper and AG-64 shows best growth with growth rate of 0.54 mm/h. Thus, by mutagenesis the ability of tolerating high copper concentrations can be increased as reported by earlier workers (Shakibaie *et al*., 2008).

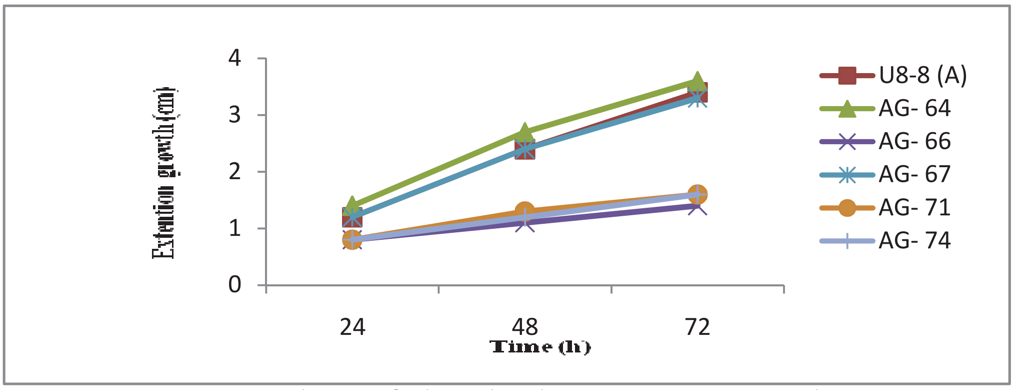

Figure 2: Extension growth rate of adapted and mutant *Neurospora* cultures at 400 ppm Cu.

All the cultures i.e. wild-type, adapted and mutant cultures were grown in Petri dishes containing 400 ppm copper and colony morphology was observed. The adapted and mutant cultures show normal colony morphology (wild-type) except AG-66 which forms small colony with irregular margin at 400 ppm copper concentration (Figure 3).

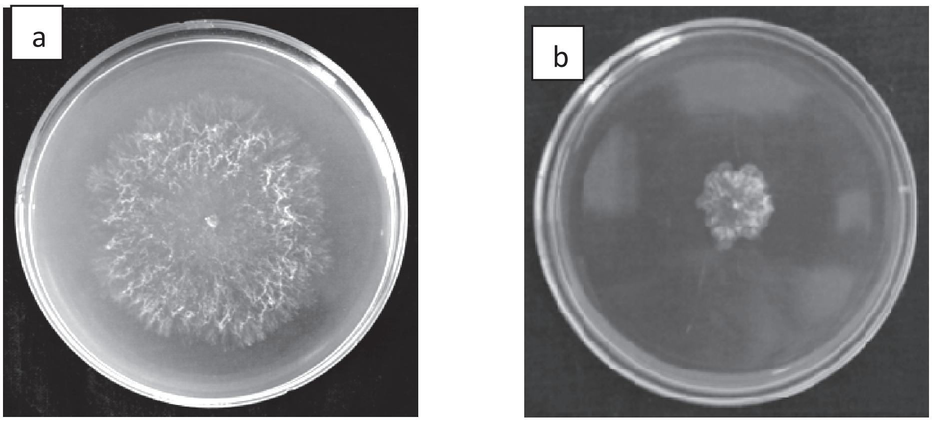

Figure 3: Colony morphology of a) wild-type (U8-8) grown on Petri dish without containing Cu in the medium and b) mutant *Neurospora* culture (AG-66) grown on Petri dish containing 400 ppm Cu in the medium.

The details of hyphal morphology were studied under microscope and results are described below (Figure 4).

U8-8A$_{Cu}$ (Wild-type culture adapted for growth on copper)**:** The culture grows well on petri plate but show frequent swollen tips and increase distance between branching. Undulated growth of hyphae at some places can be seen. Mycelium appears green at places showing accumulation of copper.

AG-64: The culture grows well on petri plate. Under microscope, it shows swelling of the tips at many places. At places bursting of tips is also seen and defect in branching is also observed.

AG-66: This culture shows growth abnormality even in petri dish. The margin of the colony is undulated. Under microscope, the culture shows condensed growth and the branches are very long and they do not form further branches. So feather like appearance is seen. The wall of the hypha at places becomes undulated instead of growing straight. At some places we could observe blue colour in the mycelium which could be due to copper accumulation and such mycelia show abnormal branching. Blue colour could also be seen in conidia which are formed by these cultures. At certain places tips show blue colour and bursting of tips at few places can be seen.

AG-67: On perti plate the culture grows fast and shows normal growth. Under microscope, reduced branching can be seen and the distance between the branching is long. At times, swollen tips are observed and dichotomous branching is seen.

AG-71: On perti plate the culture shows normal growth. Base of the branches and tips became swollen and branching abnormalities can be seen.

AG-74: The culture grows well on petri plate. The distance between branching is more. Long side branches are seen that do not branch further or have reduced branching. Branching defects can be seen. Mycelium is swollen at places. Tips of the hyphae become swollen and bursting of tips can be seen. Green colour can be seen in the mycelium.

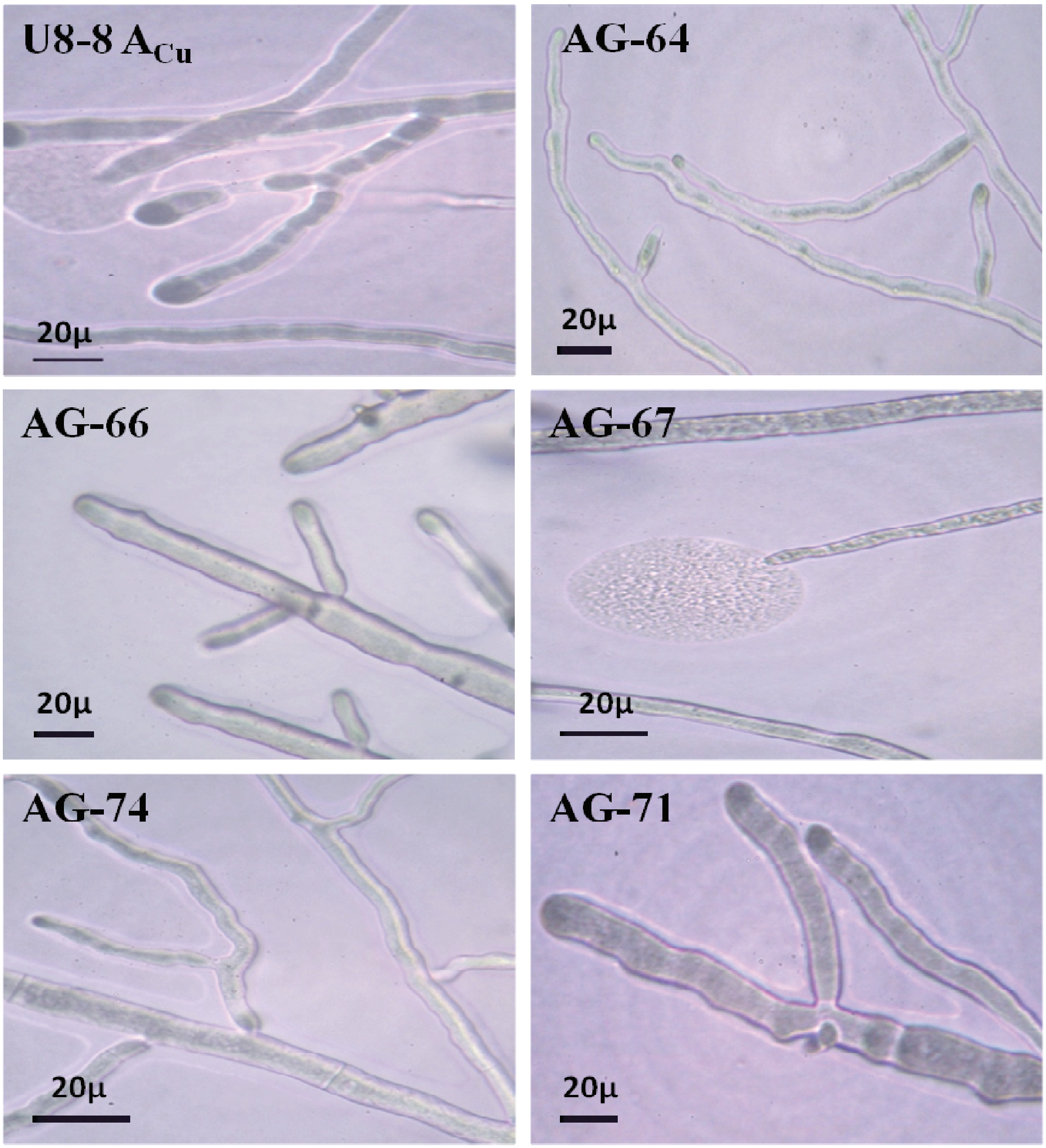

Figure 4: Hyphal morphologies of adapted and mutant *Neurospora* cultures grown in 400 ppm Cu.

Hyphal growth is essentially due to two important features which are polarized tip growth and its branching. Our study shows that at high concentration of copper, the mutants as well as adapted strains show reduced growth. This could be due to the effect of copper on hyphal tip and on branching. All the cultures under the study showed swollen tips and branching defects. Some cultures show green colour in mycelium which can be due to accumulation of copper, these cultures may be used for bioremediation of copper.

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