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Antifungal Activity of Nutrients against Mulberry Root Rot Pathogen *Macrophomina phaseolina* (Tassi.) Goid

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A B S T R A C T

In vitro evaluation of nutrients against mulberry root rot pathogen indicated that the ZnSO4 at 250, 500, 750 and 1000 ppm concentration inhibited the growth of *Macrophomina phaseolina*. The growth inhibition of 100 per cent was observed at 24, 48 and 72 h after inoculation in 1000 ppm concentration of $ZnSO_4$.

Introduction

Mulberry (*Morus* spp., Moraceae) is grown as a perennial crop for its foliage to feed silkworms (*Bombyx mori* L.). It was reported that about 60% of the cost of cocoon production accounted towards mulberry leaf production (Yokoyama, 1962, Anon., 1997). After the introduction of high yielding varieties, with large genetic diversity followed by intensive cultivation practices, mulberry has become susceptible to various diseases. Moreover, productivity and per cent disease incidence were influenced by soil nutrients (Aly *et al*., 2008). Especially root rot caused by *Macrophomina phaseolina* became severe due to continuous depletion of nutrients from the soil. This disease has developed into more alarming because of its epidemic character and propensity to kill the plant completely.

Fungicides were widely used in conventional agriculture to control soil borne plant diseases. Moreover, excessive use of chemical pesticides not only controls the disease but also might lead to the development of fungicide resistance and

caused environmental pollution (Muthomi *et al*., 2007). So there is a need to find alternatives for fungicides to manage root rot disease in mulberry without affecting the health of silkworm.

Several organic and inorganic salts were widely used for suppression of fungal pathogens on various crops (open field and protected) responsible for various diseases on different plant organs and growth stages (Deliopoulos *et al*., 2010). Importantly, these salts had a broad spectrum of antimicrobial activity with low mammalian toxicity, possess biocompatibility (Host *et al.*, 1992; Oliver *et al.*, 1998) and were generally recognized as safe (GRAS). Thus, they were widely exploited in food industry as preservatives, pH regulators and antimicrobial agents (Oliver *et al.*, 1998). Micro- and macronutrients could provide a solution to intractable and ever notorious charcoal rot disease of mung bean by inducing resistance in the plant against pathogens and growth improvement (Khan *et al*., 2018).

Nutrients were important for growth and development of plants and also microorganisms and also played an important role in disease control (Agrios, 2005). Microelements were crucial for plant growth since they not only participated in building cell walls, cell membranes (B, Zn), and enzymes (Fe, Mn, Cu) but also affected enzyme activity (Mn, Zn) and photosynthesis (Fe, Cu, Mn) (Stajic *et al*., 2012). Hence, eight nutrients were tested *in vitro* to know their inhibitory effect on the growth of *Macrophomina phaseolina* (Tassi) Goid.

Materials and Methods

Isolation of root rot pathogen

The root rot pathogen was isolated from the infected mulberry root samples by tissue segment method (Rangaswami, 1972) using Potato Dextrose Agar (PDA) medium and purified by adopting hyphal tip method (Tutte, 1969) and maintained on PDA slants at 4^{o}C for further studies.

Effect of nutrients on the growth of *M. phaseolina*

The efficacy of the following nutrients *viz*., ammonium sulphate, calcium chloride, calcium sulphate, manganese sulphate, potassium chloride, potassium nitrate, sodium chloride and zinc sulphate at 4 levels *viz*., 250, 500, 750 and 1000 ppm (w/v) were tested on mycelial growth of *M. phaseolina* under *in vitro* conditions.

Following poisoned food technique (Schmitz, 1930) the desired amounts of nutrient salts were incorporated in the molten PDA. To prepare nutrient incorporated PDA, 100 ml of PDA was taken in a sterile conical flask and mixed with 0.025, 0.05, 0.075 and 0.1 g of different nutrients to obtain a 250, 500, 750 and 1000 ppm concentration of the nutrients and the pH was adjusted to 7 by adding HCl or NaOH as per requirement. The nutrient incorporated PDA medium was poured in sterile Petri dish @ 20 ml/plate and allowed to solidify. A mycelial disc (9 mm diameter) of *M. phaseolina* was kept in the centre of Petri plate and incubated at room temperature (28±2°C). The medium without nutrients, inoculated in a similar manner, served as control. Three replications were maintained. The colony growth was recorded at 24 h interval up to 72 h after inoculation.

Results and Discussion

Studies on the effects of microelements on the growth of *M. phaseolina* under *in vitro* conditions had been consistent. Zinc sulphate at 500 ppm concentration retarded the mycelial growth and dry weight of *R. solani*

(Lakpale *et al.*, 1997). The antifungal activity of zinc sulphate against root rot was reported by Sundravadana and Alice (2006). Murugapriya *et al*. (2011) reported that zinc sulphate @ 1000 ppm concentration retarded the growth of *M. phaseolina*.

Deshmukh and Vanitha (2016) reported that zinc sulphate (0.1 %) inhibited the mycelial growth to a tune of 68.81 per cent followed by calcium nitrate and calcium carbonate each at (0.1%) recorded 50.36 and 41.80 per cent inhibition of mycelial growth over control respectively.

Zinc also plays a critical role in plant disease tolerance and should be considered as a preventive solution in disease management plans. Besides protection against diseases Zn could cause the formation of the physical barrier in plant roots against invading pathogens (Khan *et al*., 2018). Zn had a positive effect on tolerance of wheat against *Fusarium solani* root rot (Khoshgoftarmanesh *et al*., 2010). Manganese was useful in controlling a number of pathogenic diseases such as powdery mildew, downy mildew, and several other fungal diseases (Brennan, 1992; Huber and Graham, 1999 and Heckman *et al*., 2003). Similar results were found in wheat, where application of B, Mn and Zn separately increased the resistance of plants to the tan spot disease (Simoglou and Dordas, 2006). In many studies, the antagonistic effect of zinc was linked with its toxicity to pathogens (Dordas, 2008).

Zinc had direct effect on fungal growth and secondary metabolism and indirect effects on host susceptibility (Duffy, 2009). Zinc sufficient plants had been found to be more tolerant to root infection by *Fusarium* wilt than Zn deficient plants (Marschner, 1995; Streeter *et al*., 2001). This probably resulted from the fungi toxicity of Zn and the role of Zn in stabilizing the membranes of root cells (Marschner, 1995). Kishore *et al*. (2001) found that ammonium molybdate, cupric sulphate, potassium meta bisulphate and zinc sulphate showed more than 95% inhibition against mycelial growth of *Phytophthora infestans*. Copper and zinc sulphate were known to have fungicidal effects (Kishore *et al*., 2001, Ganguly *et al*., 2003). Zinc sulphate, aluminium chloride and copper sulphate were highly effective against *P. infestans* at whole plant level (Bhat *et al*., 2007).

In most cases, the application of Zn reduced disease severity, which could be because of the toxic effect of Zn on the pathogen directly and not through the plant's metabolism (Graham and Webb, 1991). The Zn-compounds treatments could be associated with altered hyphal morphology and cell damage that were not repaired conveniently (Semighini and Harris, 2010). The fungal hyphae treated with $ZnSO_4$ and Zn $(ClO_4)_2$ were observed the strongest Evans blue staining intensity indicated that these compounds inflicted higher damage to fungal hyphae (Savi *et al*., 2013). The suggested mechanism for the antimicrobial activity of Zn compounds could be based in the formation of reactive oxygen species (ROS) that disrupted the integrity of the microbial cell membrane, which assisted in the damage of microbial enzyme bodies, killed the pathogenic microbe (Feng *et al*., 2000; Applerot *et al*., 2009; Liu *et al*., 2009; He *et al*., 2011). Zn-compounds treatments could cause biochemical changes in fungal cells, disrupted cells integrity. Zn treatment lead to the death of the hyphae that undergo plasma membrane lysis. The Zn compounds treatments interfered to the fungi cellular metabolism, on conidia (number reduction) and hyphae (morphological alterations, mortality and ROS production) (Table 1).

Table.1 Effect of nutrients on the mycelial growth of *M. phaseolina* under *in vitro* condition

Nutrients	Conc. of nutrients 250 ppm				Conc. of nutrients 500 ppm				Conc. of nutrients 750 ppm				Conc. of nutrients 1000 ppm			
	Mycelial growth (mm) 24 h	Mycelial growth (mm) 48 h	Mycelial growth (mm) 72 h	Per cent inhibition over control	Mycelial growth (mm) 24 h	Mycelial growth (mm) 48 h	Mycelial growth (mm) 72 h	Per cent inhibition over control	Mycelial growth (mm) 24 h	Mycelial growth (mm) 48 h	Mycelial growth (mm) 72 h	Per cent inhibition over control	Mycelial growth (mm) 24h	Mycelial growth (mm) 48h	Mycelial growth (mm) 72h	Per cent inhibition over control
Ammonium sulphate ($(NH_4)_2SO_4$)	26.90	49.60^{b}	86.70	3.67	22.10	42.20^{de}	84.00^{cd}	6.67	16.90	35.40^{d}	77.40^{c}	14.00	6.90	26.50^{cd}	60.00^{c}	33.33
Calcium chloride ($CaCl_2$)	29.80^{d}	50.10^{b}	89.00	1.11	22.90^{d}	43.10^{e}	86.40^{cde}	4.00	18.10	37.10^{de}	79.30^{cd}	11.89	7.80	27.20^{de}	72.50^{d}	19.44
Calcium sulphate ($CaSo_4$)	28.70	49.50^{b}	88.90	1.22	19.40	38.60^{bc}	86.60^{de}	3.78	14.80	33.40^{c}	85.40^{e}	5.11	6.30^{b}	26.00^{cd}	82.00^{e}	8.89
Manganese sulphate ($MnSO_4.H_2O$)	22.90	43.40^{a}	84.30	6.33	17.50	36.60^{ab}	78.00^{b}	13.33	10.30	29.40^{b}	48.00^{b}	46.67	0.00	12.00^{b}	19.40^{b}	78.44
Potassium chloride (KCl)	29.20^{d}	50.00^{b}	89.67	0.37	24.20	44.20^{e}	88.20^{de}	2.00	19.70	38.70^{e}	83.20^{de}	7.56	11.30	30.20^{f}	78.90^{e}	12.33
Potassium nitrate (KNO_3)	28.50	49.40^{b}	86.60	3.78	19.90	40.00^{cd}	82.00^{bc}	8.89	14.50	33.40^{c}	81.40^{cde}	9.56	5.60	25.40^{c}	79.40^{e}	11.78
Sodium chloride (NaCl)	30.70	51.20^{bc}	89.54	0.51	23.40^{e}	43.50^{e}	87.30^{de}	3.00	18.30	37.60^{e}	80.30^{cd}	10.78	9.30	28.70^{ef}	74.60^{d}	17.11
Zinc sulphate ($ZnSo_4$)	20.70	42.00^{a}	83.40	7.33	15.90	35.40^{a}	66.00^{a}	26.67	7.80	21.40^{a}	28.00^{a}	68.89	0.00	0.00^{a}	0.00^{a}	100.00
Control	34.60^{f}	53.50^{c}	90.00	0.00	34.60^{g}	53.50^{f}	90.00^{e}	0.00	34.60^{g}	53.50^{f}	90.00^{f}	0.00	34.60^{g}	53.50^{g}	90.00^{f}	0.00
SEd	**0.7531**	**1.2868**	**2.3049**		**0.6063**	**1.1024**	**2.1857**		**0.4957**	**0.9415**	**1.9660**		**0.3433**	**0.7391**	**1.7558**	
CD	**1.5823****	**2.7034****	**NS**		**1.2739****	**2.3160****	**4.5921****		**1.0415****	**1.9780****	**4.1305****		**0.7212****	**1.5528****	**3.6952****	

Plate.1a Inhibitory effect of nutrients against *M. phaseolina in vitro*

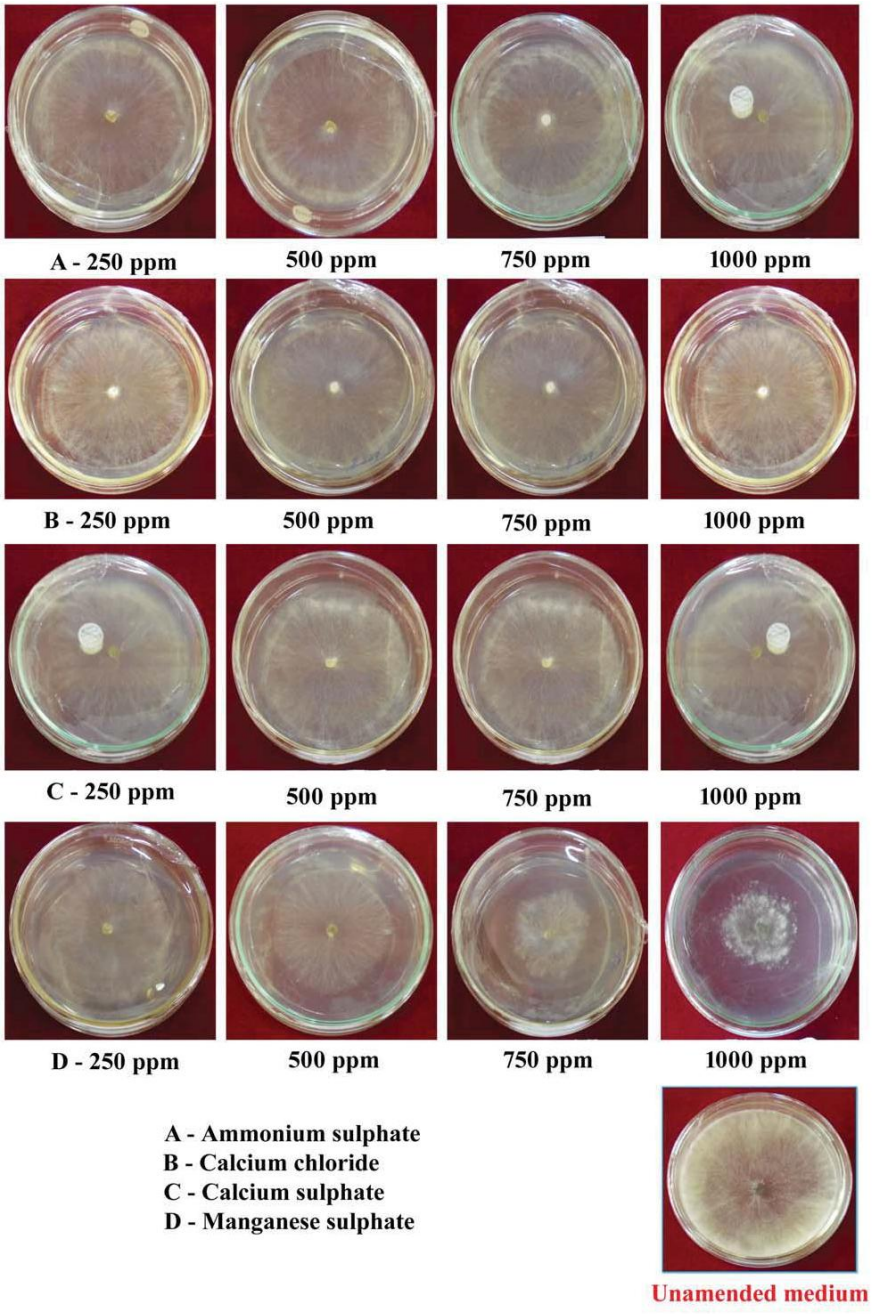
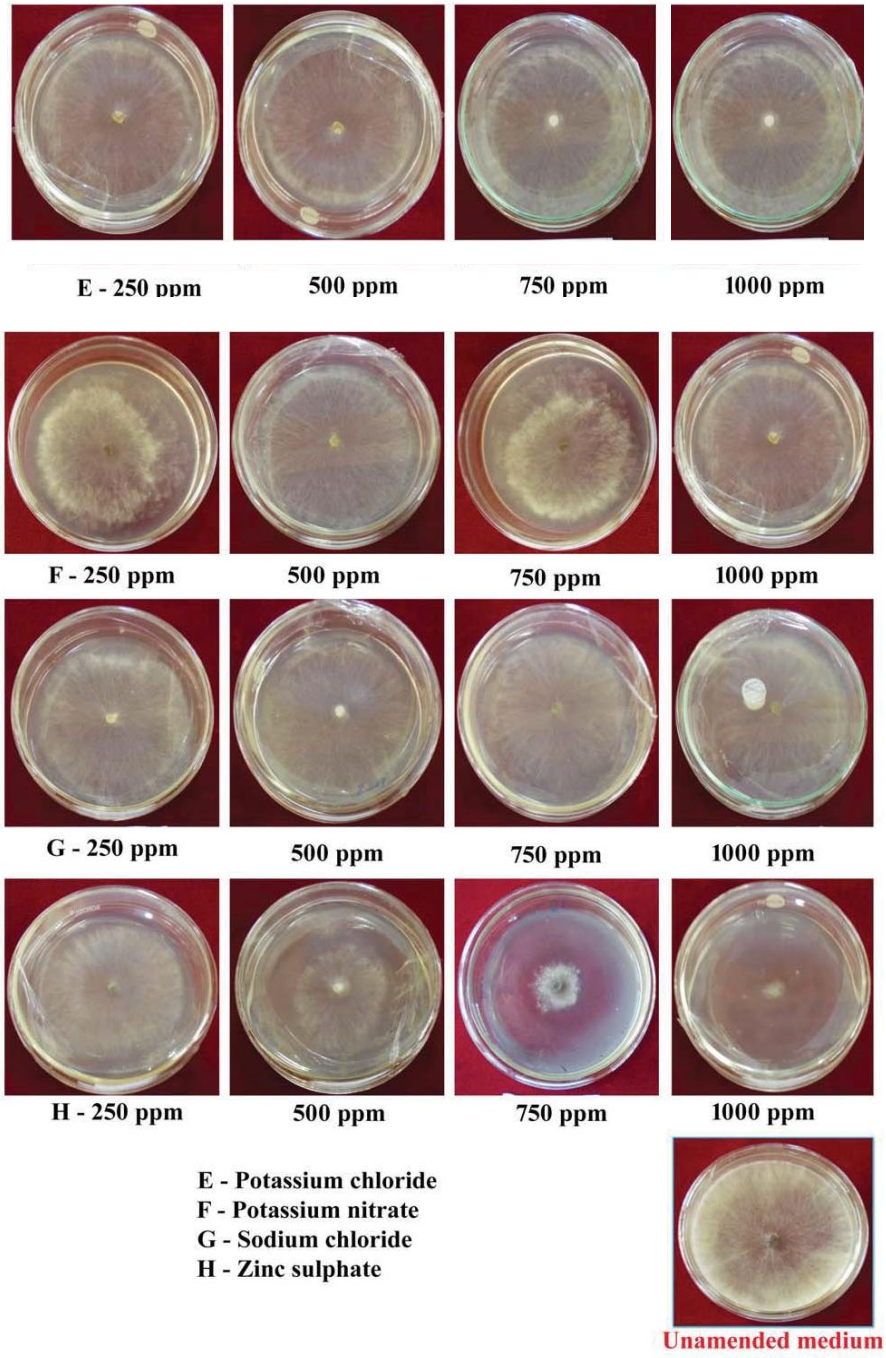


Plate.1b Inhibitory effect of nutrients against *M. phaseolina in vitro*



SEM analyses confirmed the fungi cell membrane rupture resulted in possible reduction of the enzymatic activity of the micro-organism due to Zn-compounds treatments (Savi *et al*., 2013).

The hyphal mortality could be related to cell structure damages and these alterations could increase dramatically the levels of ROS formation and be responsible for the fungi cell death (Avery, 2001). Its role had been found vital in triggering of many defense-related enzymes such as SOD, CAT and POX (Valko *et al*., 2005).

Direct effects of manganese (Mn) against pathogens included growth inhibition, reduced sporulation and toxin production for example, some strains of *Gaeumannomyces graminis* and *Magnaporthe grisea* that lack the ability to oxidize Mn exhibited decrease aggressiveness (Datnoff *et al*., 2007). Mn had an important role in lignin biosynthesis, phenol biosynthesis, photosynthesis and several other functions (Marschner, 1995; Graham and Webb, 1991).Manganese inhibited the induction of aminopeptidase, an enzyme which supplied essential amino acids for fungal growth and pectin methylesterase a fungal enzyme that degraded host cell walls (Graham and Rovira, 1984).

The results obtained in the present study corroborated with the previous findings as summarized above. Among the nutrients $ZnSO_4$ at 1000 ppm concentration completely inhibited the growth of the pathogen (*M. phaseolina*) in *in vitro* and could be exploited for the management of mulberry root rot under field condition.

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