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IN VITRO COMPARATIVE STUDY OF ANTIFUNGAL ACTIVITY OF INORGANIC SALTS AGAINST PLANT PATHOGENIC FUNGI

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ABSTRACT

Eight inorganic salts viz., ammonium molybdate, ammonium oxalate, cupric sulphate, EDTA, ferric chloride, ferrous sulphate, potassium meta bi sulphate and zinc sulphate were tested at 10mM concentration by membrane filter and steam sterilization methods against plant pathogenic fungi which damage the crop of potato(main food crop of farmers) viz., Fusarium solani, Fusarium oxysporum, Rhizoctonia solani and Phytophthora infestans. Out of these pathogens, Phytophthora infestans is major disease of potato and spreads epidemically through out the world. All chemicals are effective for R. solani and P. infestans while least effective for F. solani and F. oxysporum.

KEYWORDS: Inhibition, inorganic salts, phytopathogenic fungi, mycelial growth, membrane filter.

INTRODUCTION

Dry rot of potato caused by *Fusarium solani* is a post harvest disease which causes severe economic losses worldwide. It inflicts 5-23% loss of potato in storage condition in India (Singh *et al*., 2002). Management of this disease can be minimized by using fungicides, bioagents, inorganic chemicals and cultural practices like wound healing, use of disease free seeds and crop rotation etc. (Secor and Gudmested 1999). *Fusarium oxysporum* cause potato wilt in some parts of western Uttar Pradesh and Madhya Pradesh which loss upto 19% in heavily infected field (Singh *et al*., 2002). Several inorganic salts have been tested for managing the soil and tuber borne diseases of potato including *Fusarium oxysporum* (Singh *et al*., 2002). Palmer *et al*., (1997) investigated the effectiveness of ammonium, potassium and sodium bicarbonates against *Botrytis cinerea in vitro* and reported that all the three bicarbonates inhibited colony growth even at a low concentration of 20 mM. Campanella *et al*., (2002) evaluated the effect of 10 calcium salts in controlling *Phytophthora* root rot of citrus *in vitro* and reported that calcium oxide and calcium carbonate significantly reduced the growth of *P. nicotianae in vitro* while calcium oxide significantly reduced zoospore production and their viability. The growth of *F. solani var. coeruleum* and *F. sambucinum* was completely inhibited by ammonium phosphate, potassium carbonate, potassium bicarbonate, sodium carbonate and sodium bicarbonate salts (Mohammad Reza Ghadiri *et al*., 2013).Mills *et al*., (2004).Senapoty *et al*., (2000) studied the efficacy of cadmium chloride, ferric chloride, sodium molybdate, sodium silicate and zinc sulfate (10^{-4} M), lithium sulfate, magnesium sulfate, potassium chloride and sodium fluoride (10^{-3} M) against the seedling blast (*Magnaporthe grisea*) of rice and reported that all the tested chemicals significantly reduced the disease severity over control. Studies carried out by Hervieux *et al*., (2002) showed that several salts significantly reduced silver scurf development on potato tuber at a concentration of 0.2 M. They also reported that aluminium chloride was the only salt which reduced the silver scurf severity when applied either 2, 4 or 7 days after *Helminthosporium solani* inoculation. The effects of seed treatment with dilute solutions of

seven metal salts, cupric chloride, zinc chloride, barium sulfate, nickel chloride, lithium sulfate, ferric chloride or manganese sulfate on sheath blight (*Rhizoctonia solani*) incidence in rice were investigated in pot and field trials by Lakshikanta-Ganguly *et al*., (2003) who reported that ferric chloride, cupric chloride, lithium sulfate, barium sulfate and zinc sulfate had moderate to strong effects in managing sheath blight. Some species can cause plant pathologies leading to loss of germination, discoloration and reduction of nutritionalvalues. Moreover, they can produce mycotoxins, especially the Fusarium sp. in crops on the field and Aspergillus and Penicillium sp. during the storage (Llorens *et al*., 2004; Scussel *et al*., 2011).

MATERIALS AND MATHODS

Salts/chemicals were tested against the mycelial growth by food poison technique (Grover and Moore, 1962) by following steam and membrane filtered sterilization. In steam sterilization method, calculated amount of salts/ chemicals were added to the rye B agar medium for *P. infestans* and PDA for all remaining fungi so as to get the final concentration of 10 mM and steam sterilized at 15 psi for 15 min. After sterilization, the molten cooled medium was poured into 90 mm Petriplate and allowed to solidify. Actively growing *P.infestans* culture was cut with the help of cork borer and agar plugs of 5 mm dia. was placed at the center of each Petridish. In all, three replications were maintained for each treatment and treatments were arranged in Completely Random Block Design (CRBD). Plates were then incubated at 18 º C in a BOD incubator till the growth in control treatments fully covered the Petridish. Measurement of the radial growth in each treatment was taken on the ninth day during which the growth in control Petridish fully covered the Petridish and the % mycelial growth inhibition was calculated as:-

$$I = 100\ X\ \frac{C-T}{C}$$

Where,
I = inhibition % of fungal growth
C = Growth (mm) in control
T = Growth (mm) in treatment

Membrane filtering of salt solution was carried out using Puradisc 25 TF disposable filter device in which calculated amount of salt solution was filtered and added to rye B medium and PDA so as to get the final concentration of 10mM. Salt solution was prepared by dissolving appropriates amount of salts/ chemicals in sterile distilled water.

RESULT AND DISCUSSION

Among all Eight inorganic salts cupric sulphate showed more than 95% inhibition in the mycelial growth of *Fusarium solani, Rhizoctonia solani and Phytophthora infestans* except *Fusarium oxysporum* (83.3% inhibition). Ammonium molybdate, ammonium oxalate, zinc sulphate were less then 20% effective by membrane filter method against *F. solani*. EDTA and potassium meta bisulphite inhibited more than 95 % mycelial growth of *Fusarium oxysporum* whereas Ammonium molybdate, ferric chloride and zinc sulphate inhibited less then 50% mycelia growth of *F. oxysporum*,whereas Cupric sulphate and ferrous sulphate inhibited more then 50% mycelial growth. EDTA, cupric sulphate and potassium meta bisulphite inhibited more than 95% growth while other chemicals showed more then 80% inhibition in the mycelial growth of *Rhizoctonia solani* by membrane filter method as well as steam sterilization method. By membrane filter and steam sterilization mentods, Ammonium molybdate, cupric sulphate, potassium meta bisulphate and zinc sulphate showed more than 95% inhibition against mycelial growth of *Phytophthora infestans* while Ferric chloride is highly effective (100% inhibition) against the growth of *P.infestans* by both membrane filter as well as steam sterilization methods. Ferrous sulphate showed 100% inhibition by membrane filter method but not effective by steam sterilization method. EDTA and potassium metabisulphate cent percent inhibit the mycelial growth of *P. infestans* by steam sterilization method but by membrane filter method, both chemicals showed almost similar results (more than 95% inhibition) in the mycelial growth of *Rhizoctonia solani* as well as *Phytophthora infestans*.

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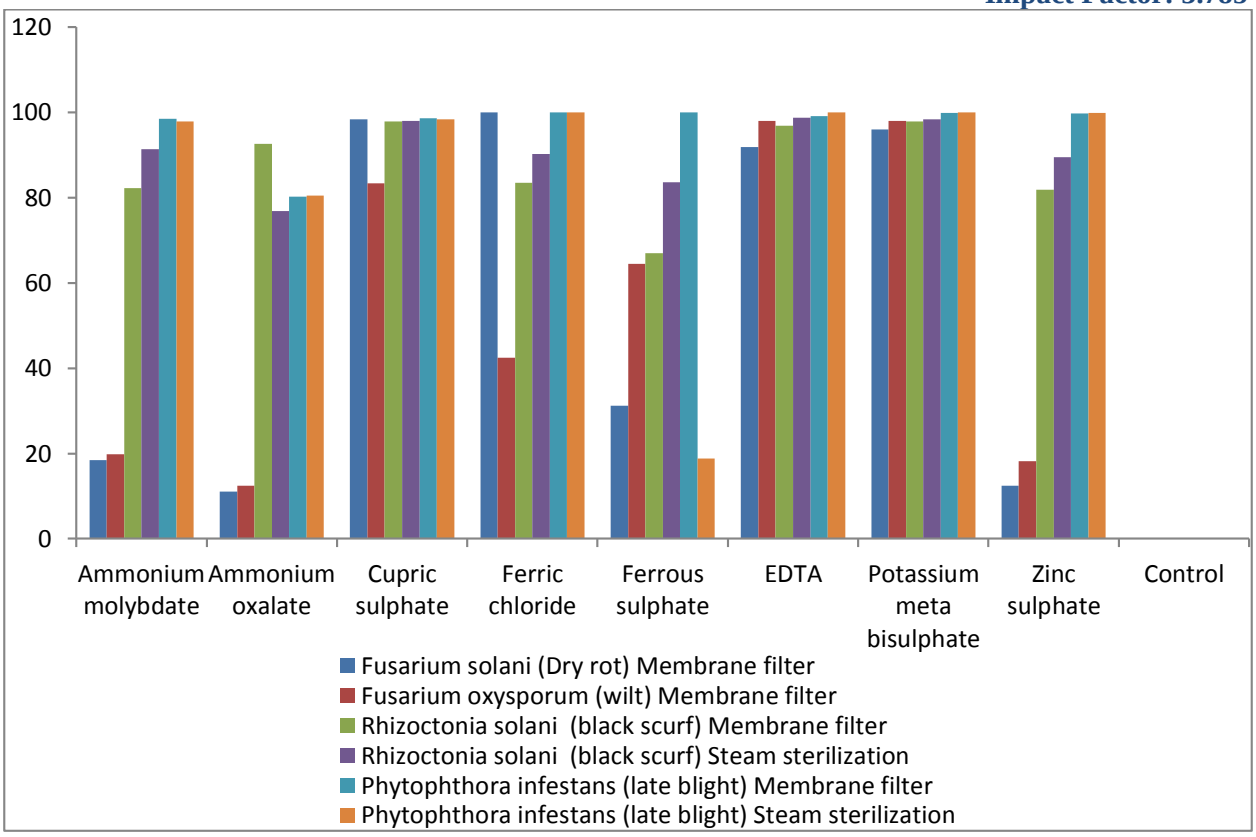

Figure: 1. Effect of inorganic chemicals against different fungi causing diseases of potato at 10mM concentration

Kishore *et al*., (2001) reported that cupric sulfate, ferric chloride and zinc chloride at 10-3 M concentration were inhibitory to both *Phaeoisariopsis personata* (conidia) and *Puccinia arachidis* (uredospores) of groundnut. Kishore *et al*., (2001) reported that cupric sulfate was effective in controlling both late leaf spot and rust in detached bioassay. Nunes *et al*., (2001) reported that ammonium molybdate inhibited the spore germination of *Penicillium expansum* and *Botrytis cinerea in vitro.* Campanella *et al*., (2002) evaluated the effect of 10 calcium salts in controlling *Phytophthora* root rot of citrus *in vitro* and reported that calcium oxide and calcium carbonate significantly reduced the growth of *P. nicotianae in vitro* while calcium oxide significantly reduced zoospore production and their viability. Mills *et al*., (2004) evaluated the effect of several organic and inorganic salt compounds and two commercial fungicides on mycelial growth, sporulation and spore germination of *Alternaria alternata*, *Botrytis cinerea*, *Fusarium solani* var. *coeruleum*, *Phytophthora erythroseptica*, *P. infestans*, *Verticillium albo-atrum*, and *V. dahliae in vitro* at 0.002, 0.02 and 0.2 M. They reported that mycelium growth and spore germination of all the pathogens were strongly inhibited by sodium metabisulfite and propyl-paraben while spore germination in most pathogens was inhibited by the aluminium compounds such as aluminium chloride, aluminium acetate and alum and the commercial fungicide mancozeb and copper sulphate. Toxic effect of potassium meta bisulphate was reported recently by Sinha *et al*., 2003.If we really solve the farmer's problems in controlling the plant diseases. Copper and zinc sulphate are known to have fungicidal effects (Kishore *et al*., 2001, Lakshikanta-Ganguly *et al*., 2003. Zinc sulphate, alluminium chloride and copper sulphate were highly effective against *P. infestans* at whole plant level. (M. Narayana Bhat *et al*., 2007). Fagundes *et al*., in 2013 also reported that among several salts the best results for reduction of gray mold *B. cinerea* on cherry tomato fruit. Bicarbonates of potassium, sodium and sodium metabisulfite showed highest antifungal activity against *Fusarium. oxysporum*, *Alternaria. alternata* and *B. cinerea* (Muharrem T and Ismail E. 2014; Masoud Zaker, 2014).

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