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ORIGINAL RESEARCH PAPER

Chemistry

IN VIVO ANTIFUNGAL BIOLOGICAL SCREENING OF SOME MIXED LIGAND TRANSITION METAL COMPLEXES AGAINST FUSARIUM WILT OF TOMATO

KEY WORDS: Metal complexes, in vitro, in vivo, Biological Screening, Antifungal.

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ABSTRACT

Metal complexes are one of the most important groups of chemical compounds and they form the basis of coordination Chemistry. Metal complexes have been growing increasing importance in the design of drugs[1,2]. The biological screening of the complexes was carried out by two different methods. These methods are in vitro and in vivo. In vitro method of biological screening was carried out under laboratory conditions and the fungal growth was measured and compared with the original growth. In vivo method of biological screening was carried out under field conditions. The present study includes the in vivo antifungal studies of synthesised mixed ligand transition metal oxalato complexes of Mn(II), Co(II), Ni(II), Cu(II) and Zn(II) with biologically important Ligand Benzoyl hydrazine on Tomato crop against Fusarium oxysporum f. Sp. lycopersici.

INTRODUCTION

Fusarium wilt of Tomato (*Lycopersicon esculentum* Mill.), caused by *Fusarium oxysporum* f. sp. *lycopersici* is one of the most prevalent, serious disease of Tomato[3]. It is also an economically important wilting pathogen of Tomato in India. The pathogen occurs throughout most Tomato-growing worldwide causing a vascular wilt that can severely affect the crop and the disease is considered as one of the main soil-borne systemic diseases[4]. It causes significant losses in tomato production both in greenhouse and field-grown Tomatoes[5,6,7]. The pathogen causes infection on leaves, stem, petiole, twig and fruits as well as leads to the defoliation, drying of twigs and premature fruit drop which ultimately reduce the yield. The disease is favoured by high temperature and humidity and plants are more susceptible to the wilt infection during fruiting period. Out of several disease management strategies the best method is chemical control.

The complexes were synthesized and characterized by elemental analysis along with spectral studies like IR, Electronic and EPR[8,9,10]. In this research paper *in vivo* chemical screening of Tomato Fusarium wilt was done by the use of synthesised transition metal complexes. The results showed that these complexes had a strong synergistic effect and could be used as a basis for a new product to control Tomato diseases.

Experimental

MATERIALS AND METHODS

Fungi And Cultures

The antifungal activities (*in vivo*) were carried out at IIVR Varanasi. The fungus *Fusarium oxysporum* f sp. *lycopersici* was obtained from the fungal collection of the laboratory of IIVR Varanasi. All strains were selected for their aggressiveness among Tomato. The pathogenic fungus was cultured and purified on potato dextrose agar medium (PDA): Extract of boiled potatoes (200 ml) dextrose(20 gm), agar(20 gm) and distilled water(1000 ml) at 280C for seven days.

In vivo studies

Tomato (*Lycopersicon esculentum*) belongs to the genus *Lycopersicon* under Solanaceae family. Tomato is a herbaceous sprawling plant growing to 1-3 m in height with weak woody stem. The flowers are yellow in colour and the fruits of cultivated varieties vary in size from cherry Tomatoes, about 1–2 cm in size to beefsteak Tomatoes, about 10 cm or more in diameter. Tomato is one of the most important "protective foods" because of its special nutritive value. It is one of the most versatile vegetable with wide usage in Indian culinary tradition. Tomatoes are used for soup, salad, pickles, ketchup, puree, sauces and in many other ways. It is also used as a salad vegetable. Tomato has very few competitors in the value addition chain of processing.

Climatic Requirements

Tomato is a warm season crop, it requires warm and cool climate. The plants cannot withstand frost and high humidity. Also light intensity affects pigmentation, fruit colour, fruit set. The plant is highly affected by adverse climatic conditions. It requires different climatic range for seed germination, seedling growth, flower and fruit set, and fruit quality. Temperature below 10^{0}C and above 38^{0}C adversely affects plant tissues thereby slow down physiological activities[11].

Soil Requirement

Tomatoes do very well on most mineral soils, but they prefer deep, well drained sandy loams. Upper layer of soil should be porous with little sand and good clay in the subsoil. Soil depth 15 to 20cm proves to be good for healthy crop. Deep tillage can allow for adequate root penetration in heavy clay type soils, which allows for production in these soil types[12s].

Tomato is a moderately tolerant crop to a wide pH range. A pH of 5.5- 6.8 is preferred. Though tomato plants will do well in more acidic soils with adequate nutrient supply and availability. Tomato is moderately tolerant to an acid soil that is pH of 5.5. The soils with proper water holding capacity, aeration, free from salts are selected for cultivation.

Soils extremely high in organic matter are not recommended due to the high moisture content of this media and nutrient deficiencies. But, as always, the addition of organic matter to mineral soils will increase yield.

Choice of seeds

After seed production, diseased, broken seeds are discarded. The seeds for sowing should be free from inert matter. Early germinating, bold, uniform in shape and size, seeds are selected for sowing. Hybrid seeds from F1 generation are advantageous for sowing as it gives early and high yield uniform fruity, resistant to adverse environmental conditions.

In vivo antifungal screening

The experiment was conducted at Indian Institute of Vegetable Research farm during crop season in randomized block design with three replications using 'DVRT-1' variety of tomato. Healthy tomato seeds were surface sterilized in 0.5% sodium hypochlorite solution for three minutes and rinsed with sterilized distilled water. The seeds were sown in sterilized soil mixed with sand 80:20 and were grown in seedling plug trays (plug size 3.4cm×3.4cm×5cm, 64 plugs). Trays were maintained under the glass house conditions at 23-280C and relative humidity 60-70%. After 21 days, plugs containing tomato plants (three true leaves) were transplanted in to plots of size 4.5×3.6 m^2 containing sterile soil infested with *F. oxysporum* f. sp *lycopersici* at a rate of 106 CFU/gm soil, and row to plant spacing was 60×45 cm2. Recommended dose of fertilizers 120:80:60 as NPK/ha respectively was applied before transplanting. All metal complexes were sprayed with same volume of solutions of

0.2% concentration on tomato plants per plot (20 plots). Control plants were similarly treated with sterile distilled water and inoculated with pathogen (without fungicides). Disease severity was recorded using 0-5 rating scale. First observation on disease severity was recorded before the beginning of first spray of fungicides, and subsequent observations were recorded before each spray. Finally, disease severity was recorded at the interval of 10 days after fungicidal spray.

In vivo antifungal activity of Metal Oxalato Complexes with Benzoyl Hydrazine on Tomato crop

Application of metal complexes reduces Fusarium wilt severity on Tomato plants inoculated under the field conditions. *In vivo* antifungal activity of Manganese, Cobalt, Nickel, Copper and Zinc metal oxalato complexes with Benzoyl Hydrazine on Tomato crop were done by spraying equal volumes of 0.2% solution of these complexes to study disease severity of wilt disease. The effects of mixed ligand metal complexes on severity of disease of Tomato crop were recorded after 10th, 20th and 30th day of spray as shown in Table- 1

Table-1 *In vivo* antifungal activity of Metal Oxalato Complexes with Benzoyl Hydrazine (different treatments) against *Fusarium oxysporum* f.sp. *lycopersici* on Tomato crop.

S. No.	Treatment	Doses	Symptoms after spraying			Infected Fruits (g/plot)	Total Yield (g/plot)	Healthy fruits (g/plot)
			10th Day	20th Day	30th Day			
1.	$Mn(OA)(BH)_2$	0.2%	++	++	+++	4875.10	40938.40	36063.30
2.	$Co(OA)(BH)_2$	0.2%	−−	++	+++	3152.25	41032.30	37880.05
3.	$Ni(OA)(BH)_2$	0.2%	++	++	+++	3853.64	47853.12	43999.56
4.	$Cu(OA)(BH)_2$	0.2%	−−	−−	−−	1402.58	78074.92	76672.34
5.	$Zn(OA)(BH)_2$	0.2%	++	++	+++	5842.52	42132.00	362.89.48
6.	Control					8628.50	18628.50	10000.00

Where
−− No symptoms
++ Leaves became slightly yellowish
+++ All folial plants wilted and became dry

In case of manganese oxalato complexes with benzoyl hydrazine, it was seen that after 10th and 20th days disease severity symptoms were less as compared to control treatment. In this condition leaves becomes slightly yellowish. Disease severity symptoms after 30th days become prominent; all folial plants wilted and became dry. Per plot total yield of tomato fruit was 40938.40 grams with infected fruits 4875.10 grams.

In case of cobalt oxalato complexes with benzoyl hydrazine, it was seen that after 10th day all the plants were healthy i.e. no disease symptoms were appeared but after 20th day some disease symptoms were appeared showing slight yellowness in the leaves and after 30th days disease symptoms were prominant showing all the wilted and dry folial plants. Per plot total yield of tomato fruit was 41032.30 grams with infected fruits 3152.25 grams.

In case of nickel oxalato complexes with benzoyl hydrazine, it was seen that after 10th and 20th days disease severity symptoms were less (slight yellowness of the leaves). Disease severity increases after 30th day. Per plot total yield of tomato fruit was 47853.12 grams with infected fruits 3853.64 grams.

In case of copper oxalato complexes with benzoyl hydrazine, it was seen that after 10th, 20th and 30th days almost all the plants were healthy i.e. no disease symptoms were recorded, leaves were green and plants were tall and healthy. Per plot total yield of tomato fruit was 78074.92 grams out of which infected fruits were 1402.58 grams.

In case of zinc oxalato complexes with benzoyl hydrazine, it was seen that after 10th and 20th day disease severity symptoms were less. Disease severity symptoms after 30th day become more with dry and wilted plants. Per plot total yield of tomato fruit was 42132.00 grams with infected fruits 5842.52 grams.

CONCLUSION

When we compare the disease severity of wilt disease by spraying these five complexes it has been found that in case of copper oxalato complexes with benzoyl hydrazine the disease severity was minimum and the weight of infected fruits was also minimum. On the basis of disease severity control these complexes may be placed in the increasing order of their antifungal action - zinc complexes, manganese complexes, nickel complexes, cobalt complexes, copper complexes.

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