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RESEARCH ARTICLE :

In vitro screening of zinc solubilizing rhizospheric isolates for agrochemicals compatibility

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SUMMARY : In the present investigation, an attempt was made to assess the compatibility of six agrochemicals such as Fungicides (Metalaxyl and Tricyclazole), Insecticides (Profenophos and Chloranthraniliprole) and Herbicides (Glyphosate and Atrazine) were tested against ten bacterial and five fungal Zn solubilizing isolates isolated from Rajendranagar, Hyderabad. Compatibility tests indicated that glyphosate has much severe effect on microbial load at low, recommended and double doses. Among insecticides Chlorantraniliprole have much more effect than Profenophos. Among fungicides Metalaxyl exerted more effect on the microbial growth, whereas among herbicides glyphosate stands out to effect microbial load. Among all isolates ZnSB-4 and ZnSF-1 are found to be susceptible for most of the agrochemicals more or less at all the concentrations.

KEY WORDS :
Zn solubilizing bacteria, Cloropyrifos, Chlorantraniliprole, Metalaxyl, Tricyclazole

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BACKGROUND AND OBJECTIVES

Soil is the most important site of biological interactions. But the extensive use of pesticides over the past four decades has resulted in tribulations caused by the interaction with natural biological system (Ayansina and Oso, 2006) conversely, sustainable agriculture involves optimizing soil resources and maintaining the quality of environment and its natural resources. One of the biggest concerns on environmental pollution is the widespread application of pesticides in agriculture. Pesticides are defined as "any substance ormixture of substances intended for preventing, destroying, repelling or mitigating any pest (insects, rodents, nematodes, fungus, weeds and other forms of terrestrial or aquatic plant or animal like bacteria or other micro-organisms)"(Mishra, 2001). Pesticide application is still the most effective and accepted means for the protection of plants from pest (Bolognesi, 2003). The pesticides that are used frequently eventually reach the soil from the crop plants and are accumulated usually in top 0-15 cm layer of soil, where the activities of microbes are found to be maximum. Pesticides in the soil affect the non target and beneficial micro-organisms (Singh and Prasad, 1991).

Agrochemical residues are generally degraded and degradation products as simulated by soil micro-organisms resulting in increased population sizes and activities of micro-organisms which in turn influences the transformations of plant nutrient elements in soil (Das and Mukherjee, 2000). On the other hand, there are some agrochemicals which are not utilizable by soil micro-organisms and these types of agrochemicals are degraded in soil by micro-organisms through co-metabolism. Other agrochemicals exert deleterious effects on micro-organisms such as interaction between agrochemicals and soil biota may be of practical significance because of possible inhibition in microbial activities contributing to soil fertility.Various studies have revealed that the herbicides can cause qualitative and quantitative change in enzyme activity (Min *et al.,* 2007; Sacki and Toyota, 2004; Sebiomo *et al.,* 2011 and Xia *et al.,* 2012). Most studies have been carried out in order to determine the effect of herbicides on soil bacterial communities and also the ability of these bacteria to degrade the herbicides (Simonsen *et al*., 2006 and Sorensen *et al*., 2008) and very little in terms of the role of fungi in the degradation and metabolism of herbicides and their effect on the community of fungi (Harms *et al.,* 2011). Therefore, no definite conclusion can be made on the effect of agrochemicals on micro-organisms and their associated transformations of nutrients in soil, since different groups of agrochemicals exhibit manifold variations in toxicity.

The present study aims to achieve a *in vitro* determination of effect of different agrochemicals (Insecticides, Fungicides and Herbicides) belonging to different groups on zinc solubilizing bacterial and fungal communities in the rhizospheric soil.

RESOURCES AND METHODS

Collection and processing of rhizosphere soil

Rhizosphere soils of different crops like Green gram, Soybean, Maize, Rice, Cotton and Sorghum were collected from a depth of 0-15 cm. Soil samples were collected from college farm and student farm at College of Agriculture, Rajendranagar, Hyderabad. Soils were shade dried for 24 hrsand removed coarse and unwanted plant debris.

Isolation of zinc solubilizing rhizospheric micro-organisms:

All the bacterial and fungal isolates were screened for their ability to solubilize zinc in TRIS minimal agar medium was amended with 0.1% of either insoluble zinc oxide (ZnO) or zinc phosphate $Zn_3(PO_4)_2$. The actively growing cultures (5 µl) was spot inoculatedonto the medium, incubated at 28 °C. Detected zinc solubilization efficiency by different rhizobacterial isolates based upon the ability of solubilization zone formation (Fasim *et al.,* 2002). The diameter of colony and clear zone around the colony was measured for calculating the solubilization efficiency in per cent and area in mm^2.

Screening for compatibility of Zn solubilizing micro-organisms with agrochemicals:

Compatibility of bacterial test isolates with commonly used agrochemicals like Fungicides (Metalaxyl and Tricyclazole), Insecticides (Profenofos and Chlorantraniliprole) and Herbicides(Atrazine and Glyphosate) (Table A) were tested by following inhibition zone technique at recommended, half recommended and double the recommonded dosages by maintaining three replications (Mohiddin and Khan, 2013).

The following fungicides, insecticides and herbicides were evaluated against zinc solubilizers under *in vitro* conditions by poisoned food technique method.

Evaluation of effect of fungicides, insecticides and herbicides on rhizospheric isolates under laboratory conditions :

Efficacy of two fungicides, two insecticides and two herbicides (Table A) were tested against rhizospheric bacteria and fungi under *in vitro* conditions by poisoned food technique at three concentrations, *i.e.*, half, recommended and double doses.For each treatment, 100 ml of nutrient agar/potato dextrose medium was taken in

Table A: Details of the fungicides, insecticides and herbicides used in bioassay studies under *in vitro* condition

Sr. No.	Fungicides	Recommended Dosages (ppm)	Insecticides	Recommended dosages (ppm)	Herbicides	Recommended dosages (ppm)
1.	Metalaxyl	200	Profenophos	200	Atrazine	2000
2.	Tricyclazo-le	60	Chlorantra-niliprole	40	Glyphosate	1000
3.	Control (Without fungicide)					

250 ml conical flask and sterilized in an autoclave. To the sterilized medium, fungicide/insecticide/herbicide was added at lukewarm temperature and mixed thoroughly by shaking to obtain required concentrations. The poisoned medium was equally distributed in the Petri plates and allowed to solidify (Nene and Thapliyal, 1993).

Three replications were maintained for each treatment whereas, for fungal cultures evaluation, Discs (5 mm) of actively growing test fungal cultures were cut with sterilized cork borer and transferred to the center of the poisoned medium in each of the Petri plates. Similarly, control was maintained by placing 5 mm discs of test fungal culture in center of the plates containing the medium without fungicide. For bacterial isolates a volume of 0.1 ml containing 10^7 cells ml^{-1} was taken with help of micropipette and transferred on to the poisoned medium. The transferred medium was spreaded equally with help of sterile spreaders. All the Petri plates were incubated at 28 ± 2 ºC in BOD incubator. The bacterial count will be taken after 2 - 4 days of incubation. For fungal isolates the diameter of fungal colony was measured in each of the treatment when the fungal colony growth in control plate was full. The colony diameter inhibited in fungicide treated plates as compared to control was taken as a measure of fungi toxicity. Per cent inhibition over control was calculated by following the equation:

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

where,

I = Per cent inhibition of mycelial growth

C = Radial growth of pathogen in control (mm)

T = Radial growth of pathogen in treatment (mm).

OBSERVATIONS AND ANALYSIS

The results obtained from the present study as well as discussions have been summarized under following heads:

Effect of fungicides on zinc solubilizing isolates

The isolates were screened for their compatibility with the two commonly used Fungicides (Metalaxyl and Tricyclazole). Among ten bacterial and five fungal isolates ZnSB-1, ZnSB-2, ZnSB-3, ZnSB-5, ZnSB-6, ZnSB-7, ZnSB-8, ZnSB-9 and ZnSB-10 exhibited good compatibility (0 mm inhibition) with fungicides atrecommended dosages (Table 1, Fig. 1 and 2). At higher doses of Tricyclazoleall the isolates showed less compatibility. With double doses of Metalaxyl all most all exhibited less compatibility except ZnSB-1, ZnSB-2,

Table 1 : Compatibility study of different Zn solubilizing isolates with insecticides, fungicides and herbicides

Isolates	Insecticides						Fungicides						Herbicides					
	Profenophos			Chlorantraniliprole			Metalaxyl			Tricyclazole			Atrazine			Glyphosate		
	*L	*N	*D	*L	*N	*D	*L	*N	*D	*L	*N	*D	*L	*N	*D	*L	*N	*D
ZnSB-1	H	H	H	H	H	H	H	H	H	H	H	L	H	H	L	L	L	L
ZnSB-2	H	H	H	H	H	H	H	H	H	H	L	L	H	H	MD	L	L	L
ZnSB-3	H	H	H	H	H	H	H	H	H	H	MD	L	H	H	H	H	H	L
ZnSB-4	H	MD	L	H	H	H	L	L	L	L	L	L	H	L	L	L	L	L
ZnSB-5	H	H	H	H	H	H	H	H	H	H	MD	L	H	H	MD	L	L	L
ZnSB-6	H	H	L	H	H	H	H	H	L	L	L	L	H	H	H	L	L	L
ZnSB-7	H	H	H	H	H	H	H	H	H	H	H	L	H	H	L	L	L	L
ZnSB-8	H	H	H	H	H	H	H	H	H	H	H	L	H	H	L	L	L	L
ZnSB-9	H	H	H	H	H	H	H	H	L	H	H	L	H	H	L	L	L	L
ZnSB-10	H	H	L	H	H	H	H	H	L	H	H	L	H	H	H	H	H	L
ZnSF-1	H	H	L	L	L	L	MD	L	L	H	MD	L	L	L	L	H	L	L
ZnSF-2	H	H	L	H	MD	L	MD	L	L	MD	MD	L	H	MD	L	MD	L	L
ZnSF-3	H	MD	L	H	MD	L	MD	L	L	MD	MD	L	H	H	L	H	MD	L
ZnSF-4	MD	MD	L	H	MD	L	MD	L	L	MD	MD	L	MD	MD	L	H	MD	L
ZnSF-5	H	H	L	H	MD	L	MD	L	L	H	MD	L	H	MD	L	H	MD	L

H- Highly compatible (>300 CFU/ plate) MD- Moderately compatible (100-300 CFU/plate)
L- Less compatible (< 100 CFU/plate) *L- Low dose *N- Recommended dose *H- High dose

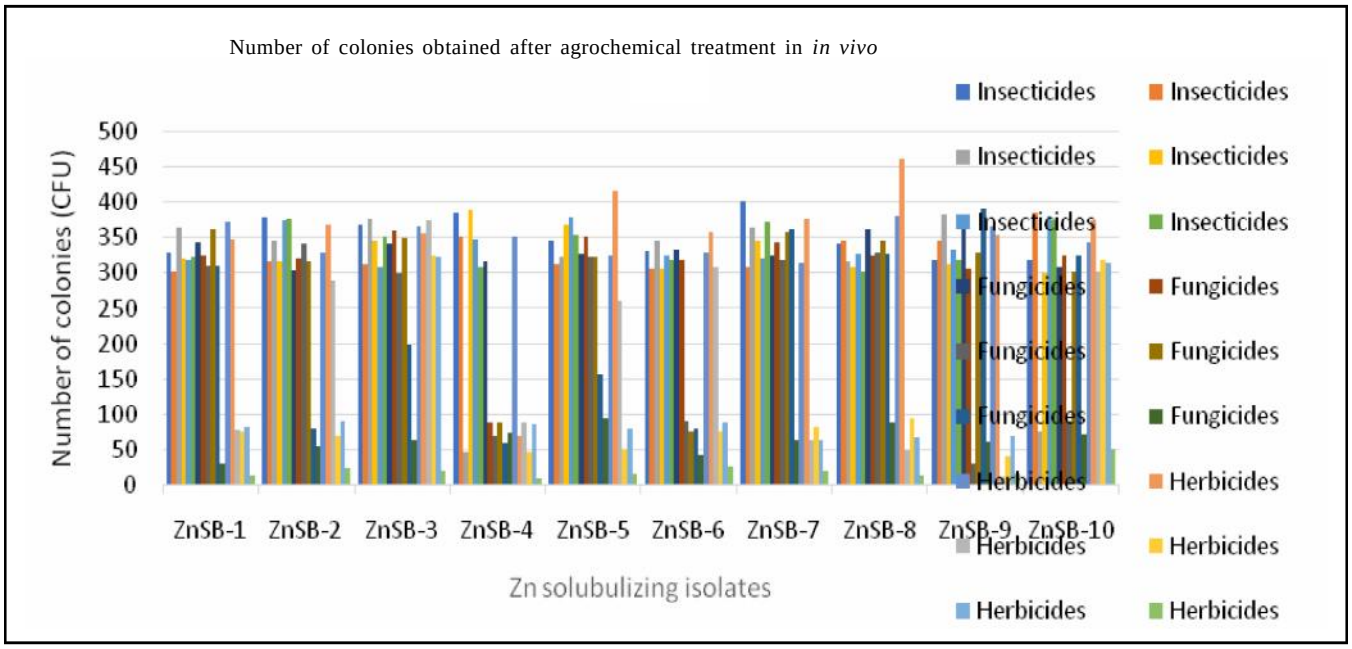

Fig. 1 : Growth pattern of Zn solubilizing bacteria in different concentrations agrochemicals inoculated plates

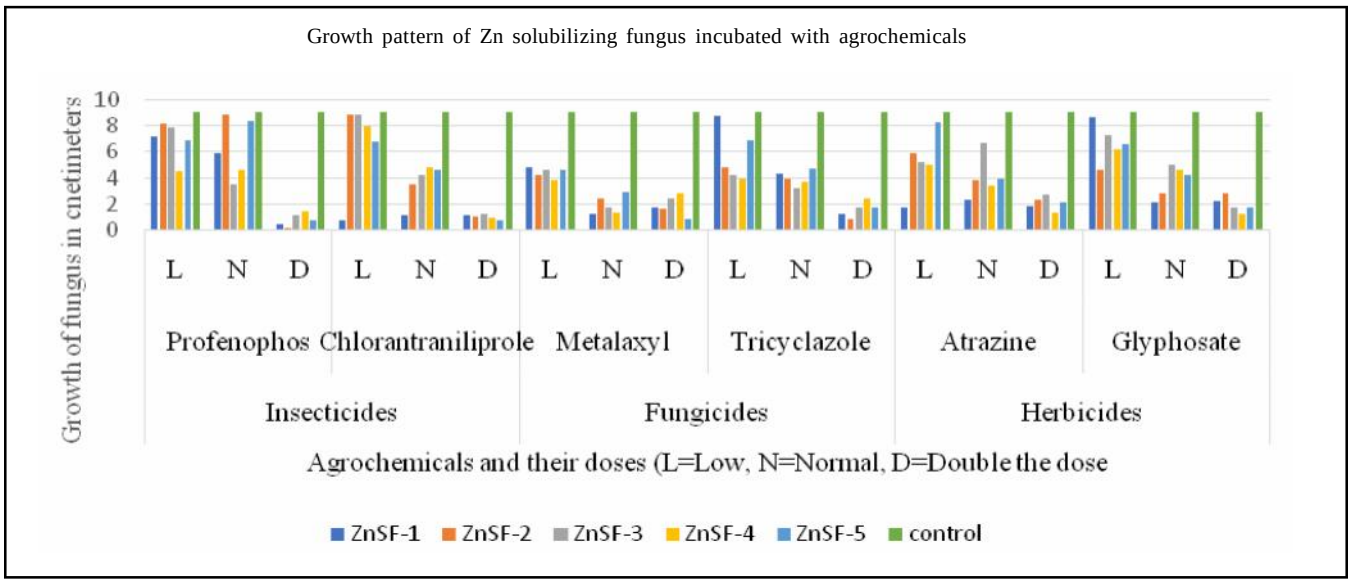

Fig. 2 : Growth pattern of Zn solubilizing fungus at different concentrations agrochemicals inoculated plates

ZnSB-3, ZnSB-5, ZnSB-7 and ZnSB-8. Among all isolates ZnSB-4 was susceptible for low, recommended and double doses *i.e.*, they are highly susceptible species.

Sharvani (2011) reported similar results who studied the *Pseudomonas, Bacillus, Rhizobium* compatibility studies against commonly used agrochemicals like Fungicides (Copper oxy chloride, Carbendazim, Thiram and Captan) Insecticides (Phorate, Carbofuran, Imidachloprid and Chlorpyriphos) and herbicides (Alachlor, Butachlor, Pendimethalin and Oxyfluorofen) at their recommended and half recommended dosages. Ahmed *et al.* (2007) reported the effect of different concentrations (0, 10, 20, 50, 100, 200, 500 and 1000 µg L^{-1}) of the fungicides Captan, Thiram, Luxan, Fernasan-D and Milcurb on inhibition of growth and colony sizes of seven *Rhizobium* strains. All strains were tolerant to

low fungicide concentrations but they were sensitive to high concentrations.

Effect of insecticides on zinc solubilizing isolates:

The isolates were screened for their compatibility with the two commonly used insecticides (Chlorantraniliprole and Profenophos). Isolates were not affected by any insecticides at recommended and low doses, whereas at double dose ZnSB-1, ZnSB-2, ZnSB-3, ZnSB-5, ZnSB-7, ZnSB-8 and ZnSB-9 were susceptible at higher concentration of profenophos (Table 1 and Fig. 1 and 2). Isolates of ZnSF-1, ZnSF-2, ZnSF-3, ZnSF-4 andZnSF-5 were affected by Chlorantraniliprole insecticide with double dosage. Pesticide soil treatment did not affect the abundance of culturable phosphate solubilizing bacteria Maria *et al*. (2015). Chennappa *et al*. (2014) found that effect of Pendimethalin, Chloropyrifos, Glyphosate and Phorate on nitrogen fixation, indole acetic acid, gibberellic acid was less and phosphate solubilizationat low concentration of Metalaxyl all isolates were highly compatible.

Similar results were found by Neeraja *et al*. (2014) who studied Carbendazim (0.2%), Chlorpyriphos (0.25%), Pendimethalin (0.66%) and zinc sulphate (0.2%) under pot culture experiment by using chickpea. Soumik *et al.* (2010) reported that systemic fungicides, Hexaconazole was the most toxic to *Trichodermaharzianum* which was, followed by Propiconazole and Triflumizole.

Effect of herbicides on zinc solubilizing isolates :

The isolates were evaluated for their compatibility with the two commonly used herbicides (Atrazine and Glyphosate). All most all isolates showed high compatibility (0 mm inhibition zone) with atrazine except, ZnSB-4 and ZnSF-1 *i.e.*, they were less compatible with atrazine. Isolates ZnSB-3 and ZnSB-10 were not affected by glyphosate which showed high compatibility (0.0 mm inhibition zone) with herbicide at all concentrations. Only ZnSF-3, ZnSF-4 andZnSF-5 isolates were moderately affected by Glyphosate herbicide (Table1).

Ebtesam *et al*. (2013) isolated Atrazine-resistant soil bacteria from different contaminated soils. Maheswari (2013) found that *Pseudomonas* isolates with systemic and non-systemic fungicides. Hefnawy *et al.* (2012) concluded that *Aspergillus niger* and *Aspergillus fumigatus* were not affected at lower concentrations of tested herbicides *i.e.* Glyphosate and Putraline upto 200 mg l^{-1} and at 800 mg l^{-1}, Madhaiyan *et al.* (2006) reported that insecticides Monocrotophos, Lindane and Dichlorvos had the most lethal action against *Gluconacetobacter* grown on LGIP medium, while Endosulphan, Chlorpyriphos, and Malathion effects were intermediate. Herbicides generally appeared to have no adverse effect on the growth and survival of *Gluconacetobacter* in the medium except for the concentrations exceeding recommended rates.

Conclusion:

In this study, the observed trends in microbial population were similar to observations made by Korpraditskul *et al*. (1988). The application of agrochemicals under *in vitro* conditions shown a significant drop in microbial load with respect to untreated control. Ayansina and Oso (2006) discovered that higher concentrations of herbicides treatments resulted in much lower microbial counts when compared to soils treated with recommended doses. Experiments have shown that microbes may use herbicides as a source of carbon (Radosevich *et al.,* 1995).Some of the agrochemicals tested delayed colonies formation from 20 to 48 h incubation. Among all agrochemicals glyphosate have severe effect on microorganism growth at all concentrations after atrazine. Most of the agrochemicals that are used in this experiment were compatible with micro-organisms and they won't affect the growth at ordinary or optimal conditions, though their persistence in soil at to be tested.

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