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The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2020; 9(7): 442-446

www.thepharmajournal.com
Received: 13-05-2020
Accepted: 15-06-2020

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Effect of pesticides on growth kinetics and plant growth promoting activities of biofertilizer

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Abstract
The present study was conducted to assess the effect of selected pesticides at recommended dosage and higher concentrations on growth and plant growth promoting activities of nitrogen fixing biofertilizer culture *Azotobacter*. The growth kinetics of the culture were least affected by recommended concentrations of pesticides. Even, the application of various pesticides at recommended rates had no deleterious effect on the plant growth promoting activities (PGP) of *Azotobacter* as well. But, enormously high concentration of pesticides brought a progressive decline in the growth and PGP activities of the culture like high concentration of chlorpyrifos significantly lowered the IAA production of *Azotobacter*. Generally maximum inhibition to the growth and PGP activites of the *Azotobacter* was reported under insecticidal influence. Therefore, this study revealed an additional aspect of pesticide effect on growth and PGP activities of microbes.

Keywords: Biofertilizers, growth kinetics, pesticide, plant growth promoting activities

1. Introduction

Soil relies intensively on external nutrient inputs to run the energy driven processes of terrestrial ecosystem. Various soil microorganisms like *Azotobacter*; *Bacillus*; *Pseudomonas*, play a crucial role in supplying major essential nutrients to the soil viz nitrogen (N), phosphorous (P) and potassium (K). These organisms, also known as plant growth promoting rhizobacteria (PGPR), stimulate the growth of plants either through direct or indirect mechanisms. Direct mechanisms include mineralization of organic matter, fixation of atmospheric nitrogen, p-solubilization, production of iron sequestering compounds (siderophores) and production of plant growth promoting hormones. Indirect mechanisms comprise HCN production, degradation of noxious compounds, inhibition of disease causing agents etc [1]. The beneficial properties of these microorganisms have convinced the agriculturists to employ their live or latent formulations as biofertilizers in the fields. *Azotobacter* is a group of free living, aerobic bacteria which converts atmospheric nitrogen into ammonium ions and makes it assimilable by the plants. It contains nitrogenase enzyme which brings about this conversion and fixes atmospheric nitrogen [2]. Various species of *Azotobacter* have been used as biofertilizer to improve the yields of enumerable crops like wheat, paddy, maize, pea etc. These bio-inoculants give promising results when used in combination with other agrochemicals under specified concentrations [3].

Pesticides are inevitable tools of agriculture involved in plant protection and production. These chemicals and their degradation products interact with the rhizospheric microorganisms both positively and negatively. However, the outcome depends upon the type of pesticide used, its concentration applied to the field and the persistence level in soil [4]. Pesticides undergo biodegradation, adsorption, desorption, transportation etc. in soil. Excessive application of pesticide brings about damage to the microflora of soil and degrades the fertility of land. Some microorganisms use the pesticide as a source of carbon and nitrogen and therefore are benefitted with the application of these chemicals at recommended rates. The degradation products provide energy to the microbes and further help in enhancement of nutrient mineralization processes in soil. Nevertheless, the detrimental effects of pesticides on microbes cannot be ignored. The unfavourable effects of pesticides include inhibition of nitrogen fixation and various other processes in soil. It has been speculated that the chemicals break the association of plant with the rhizobacteria and therefore bring about the inhibiton of nitrogen fixation.

The biofertilizers employed in the fields display high tolerance to the pesticides and therefore could be used in conjunction with the pesticides.

These bio-inoculants can perform various PGP activites under pesticide stress and enhance plant growth in the field [5]. The present study was conducted to assess the minimum inhibitory concentrations of six pesticides (chlorpyrifos, raxil and isoproturon) towards the recommended biofertilizer i.e. *Azotobacter spp*. (recommended for wheat crop by Punjab Agricultural University, Ludhiana in conjuction with two other cultures) and to further study the effect of different concentrations of pesticides on PGP activities of the culture under lab conditions.

2. Materials and Methods

2.1. Biofertilizer culture (Azotobacter spp.) and pesticide tolerance

The culture was tested for its compatibility with three different groups of pesticides as mentioned in Table 1, namely; insecticides viz. Chlorpyrifos, fungicides viz. Raxil, herbicides viz. Isoproturon by agar plate dilution method and disc diffusion method using minimal salt agar medium (g/l : KH_2PO_4 1; K_2HPO_4 1; NH_4NO_3 1; $MgSO_4.7H_2O$ 0.2; $CaCl_2.2H_2O$ 0.02; $FeSO_4.7H_2O$ 0.01 and pH 6.5. The freshly prepared plates were amended with different concentrations of pesticides to determine the growth of cultures at varying concentrations of pesticides. The minimum inhibitory concentration of pesticides was determined using disc diffusion method. The discs were previously sterilized and placed on surface of the medium containing inoculated culture of biofertilizer. Triplicates were made of each plate to maintain accuracy. A pesticide free disc was placed as a control. Minimum inhibitory concentration of pesticides was determined on development of zones of inhibition around the discs.

Table 1: Pesticides used in the study

Category	Common name	Chemical name	Chemical family	Recommended dose for wheat crop
Insecticides	Chloropyrifos	O,O-diethyl O-3.5.6-trichloropyridin-2-yl phosphate	Organophosphate	4 ml/kg seed
Fungicide	Raxil	(rs)-1-(4-chlorophenyl)-4,4-dimethyl-3-(1h,1,2,4-triazole-1-ylmethyl)pentan-3-ol	Tebuconazole	1g/kg seed
Herbicide	Isoproturon	3-(4-isopropylphenyl)-1,2-dimethylurea	Triazolinone	500 g/acre

2.2. Growth patterns of culture under different concentrations of pesticide

The growth kinetics were determined using calorimetric analysis of freshly inoculated culture (0.1 ml) in 10 ml mineral salt medium containing different concentrations of pesticides. A flask containing no added pesticides was kept as a control. The cultures were incubated for four days at 28± 2°C on rotary shaker. The absorbance of cultures was noted at regular time intervals at 540 nm using a spectrophotometer. The growth curves were obtained by plotting optical density as a function of time.

2.3. Plant Growth Promotion Activities

The plant growth promotion activities of the *Azotobacter* culture were assayed to check the effect of pesticides on it. The quantitative estimation of Indole Acetic Acid (IAA) by the biofertilizer in presence of different doses of pesticides was done by method given by Gordon and Weber [6]. The method of Lata and Saxena [7] was used to assay ammonia production of the biofertilizer cultures in the presence and absence of different pesticides. The estimation of ammonia excreted in supernatant was carried out with the Indophenol method given by Bergersen [8]. The qualitative test for siderophore production was carried out on Chrome Azurol S agar (CAS) by the method given by [9].

3. Results and Discussions

3.1. Pesticide tolerance

Various agro-chemicals used in the study displayed wide variation in terms of their inhibitory capacities towards the *Azotobacter* biofertilizer as mentioned in Table 2. The MIC values ranged between 16-1000 mg/l. The toxicity pattern of pesticides towards biofertilizer was chlorpyrifos> isoproturon> raxil. Similarly, Khan *et al.* [10] reported that the MIC values of *Bradyrhizobial* isolates towards herbicides (isoproturon, atrazine, metribuzin and sulfosulfuron) ranged between 3200 to 6400 µg/ml.

Table 2: Minimum inhibitory concentrations of the pesticides used in study

Category	Names of Pesticides	Recommended Dose	Culture A MIC* (mg/l)
Insecticide	Chlorpyrifos	4 ml/ kg seed	16
Fungicide	Raxil	1g/ kg seed	1000
Herbicide	Isoproturon	500 g/ acre	990

where MIC* represents the minimum inhibitory concentration of pesticides

3.2. Growth kinetics in the presence of pesticides

The insecticide used in the study (Chlorpyrifos) had no negative effects on the growth kinetics of *Azotobacter* at field rates (4ml/kg seed). However, the growth curve showed a significant inhibition in presence of higher concentration (8 mg/l) of insecticide (Figure 1). The results reported are in accordance with study conducted by Supreeth *et al.* [11] where they reported deleterious effect of chlorpyrifos on colony forming units of bacteria in soil.

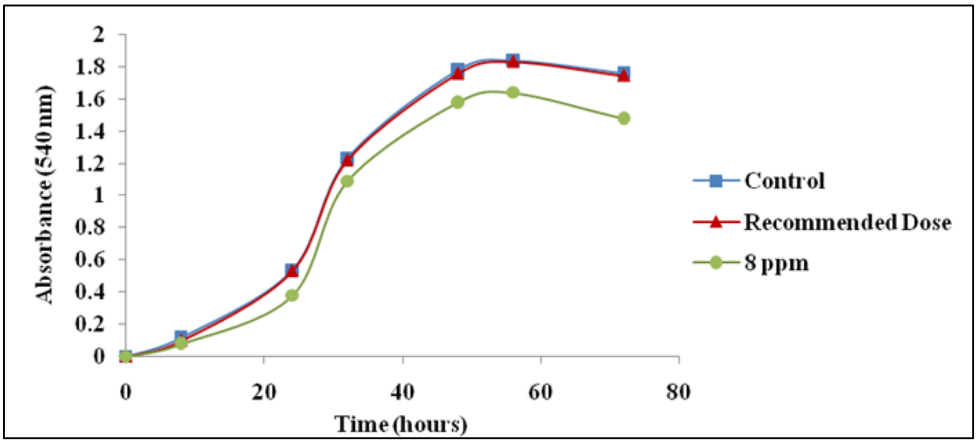

Fig 1: Effect of different concentrations of Chlorpyrifos on growth kinetics of *Azotobacter*

Raxil (Fungicide) was probably used as a source of nutrition by culture as observed by the rising trend in growth curve (Figure 2) of culture under the fungicidal influence. Nowak *et al.* [12] also noted stimulatory effects of fungicides on bacteria. However, Azotobacter was inhibited by 495 ppm concentration of herbicide i.e. isoproturon (Figure 3).

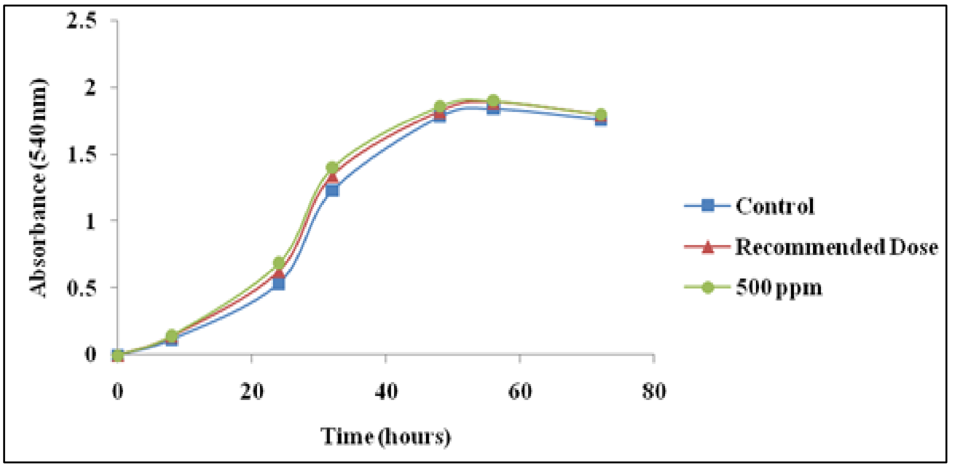

Fig 2: Effect of different concentrations of raxil on growth kinetics of *Azotobacter*

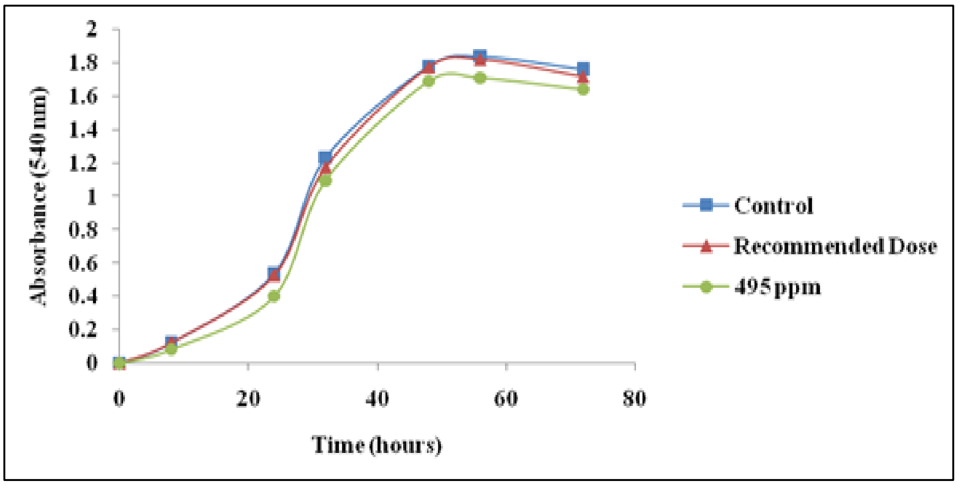

Fig 3: Effect of different concentrations of isoproturon on growth kinetics of *Azotobacter*

3.3. Indole acetic acid production in the presence of pesticides

IAA is the phytohormone produced by some beneficial bacteria for the growth promotion of plant [13]. All of the agro-chemicals used in the study reported no significant negative impact on IAA production of the cultures at the recommended doses. However, doses above the field rates had variable effect on the IAA production of the cultures. Higher dose of chlorpyrifos reduced IAA production in the culture remarkedly as only 20.40µg/ml of IAA was produced in the presence of chlorpyrifos (Table 3). Similarly, the herbicide isoproturon also reduced the IAA production in culture A (25.20µg/ml). The stimulatory effect of raxil on culture A was insignificant at higher dose (30.90µg/ml). The MIC doses of the pesticides completely exhausted the IAA production capacity of culture.

On the whole, the recommended doses of the pesticides had no adverse effect on the IAA production by the biofertilizer. This is well in accordance with the study conducted by Kulandaivel and Nagarajan [14] where they observed that the field rates of pesticides had little effect on the IAA production of rhizobacteria.

Table 3: Effect of different doses of pesticides on IAA production of *Azotobacter* in absence and presence of tryptophan

IAA production (µg/ml)								
Category	**Pesticides used**	**Doses (µg/ml)**	**Without Tryptophan**			**With Tryptophan**		
			Incubation Period		**Mean**	**Incubation Period**		**Mean**
			3 Days	**5 Days**		**3 Days**	**5 Days**	
Control	No Pesticide	0	12.50	20.95	16.73	21.00	39.60	30.30
Insecticides	Chlorpyrifos	R*	11.90	19.60	15.75	20.50	38.78	29.64
		8	06.10	11.50	08.80	13.40	27.40	20.40
		16	00.00	00.00	00.00	00.00	00.00	00.00
Fungicides	Raxil	R*	12.40	21.90	17.15	21.50	39.40	30.45
		500	12.20	21.80	17.00	21.70	40.10	30.90
		1000	00.00	00.00	00.00	00.00	00.00	00.00
Herbicides	Isoproturon	R*	12.04	21.20	16.62	20.50	39.10	29.80
		495	08.20	17.01	12.61	17.60	32.80	25.20
		990	00.00	00.00	00.00	00.00	00.00	00.00
CD@5%			0.22			0.67		

3.4. Production and excretion of ammonia in the presence of pesticides

Ammonia synthesis by various rhizobacteria act as signaling source for interaction between the bacteria and the plant [15]. Biofertilizer culture produced substantial amount of ammonia. The supplementation of media with pesticides at concentrations equivalent to their recommended doses had no negative effect on ammonia excretion by the culture. However, elevated doses of chlorpyrifos (7.5-8 ppm) lowered the ammonia excretion of the culture to 2.7µg/ml. The higher dose of raxil (300 ppm) promoted ammonia excretion of culture. However, ammonia excretion of *Azotobacter* culture (3.15µg/ml) was negatively affected by 495 ppm concentration of isoproturon (Table 4). MIC doses of the pesticides completely depleted the ammonia production and excretion capacity of the biofertilizer under lab conditions.
The results find support in the observations made by Rani *et al.* [16] where the researchers observed that higher concentrations (above recommended dose) of organochlorine pesticides like lindane, aldrin and endosulfan did not completely inhibit the ammonia production capacity of the *Paenibacillus* sp. IITISM08.

Table 4: Ammonia production and ammonia excretion by *Azotobacter*

Category	**Pesticides used**	**Doses (µg/ml)**	**Ammonia production**	**Ammonia excretion(µg/ml)**
Control	No Pesticide	0	+	4.30
Insecticides	Chlorpyrifos	R*	+	4.21
		8	+	2.70
		16	-	0.00
Fungicides	Raxil	R*	+	4.37
		500	+	4.32
		1000	-	0.00
Herbicides	Isoproturon	R*	+	4.27
		495	+	3.15
		990	-	0.00
CD@5%			0.49	

R*represents recommended doses of pesticides

3.5. Production of siderophores in the presence of pesticides

Siderophores are low molecular weight iron chelators which are produced by the microbes under iron deficit conditions. The production of siderophores was observed in the presence as well as absence of pesticides. Recommended doses of pesticides had no negative effect on siderophore production of cultures. Similar results were reported by other scientists where they observed no inhibitory effect of pesticides on *Mesorhizobium* strain LGR2 used as biofertilizer. However, the inhibitory doses of pesticides lead to exhaustion of siderophore production capacity of the cultures.

3.6. Phosphorus solubilization in the presence of pesticides

Phosphorous solubilisation by microbes is desirable character for promotion of plant growth [19]. As observed in case of other PGP traits, p-solubilization was not affected by the pesticides at recommended rates. More than recommended dosage of pesticides yielded variable effects on p-solubilization capacity of *Azotobacter* culture. Amongst the three pesticides, chlorpyrifos (7.5-8 ppm) was found to damage the p-solubilization capacity of culture. Raxil was apparently stimulatory for p-and 495 ppm concentration of the isoprutoron reduced the p-solubilization capacity of culture from 10.74µg/ml to 7.26µg/ml (Table 5). Similar results were reported by Ramani and Patel [20].

Table 5: Effect of different doses of pesticides on P-solubilization activity of *Azotobacter*

Category	Pesticides used	Doses (µg/ml)	Phosphate solubilization (clear zone formation)	Phosphate solubilization activity (µg/ml)
Control	No Pesticide	0	+	10.74
Insecticides	Chlorpyrifos	R*	+	10.69
		8	+	6.24
		16	-	00.00
Fungicides	Raxil	R*	+	10.82
		500	+	10.16
		1000	-	00.00
Herbicides	Isoproturon	R*	+	10.63
		495	+	7.26
		990	-	00.00
CD@ 5%				0.22

R*represents the recommended doses of the pesticides

4. Conclusion

The present investigation concluded that the application of pesticides at field rates had no negative effect on the growth kinetics of *azotobacter* culture. Even the plant growth promotion activities of biofertilizer were not affected in presence of pesticides. As biofertilizer application enhance the fertility of the soil and thus these results ensure that the beneficial effects of *azotobacter* will not be effected application of pesticides in crop. Hence even the combined application of biofertilizer and pesticides at recommended rate satisfies the criteria of sustainability.

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