

Original Research Article

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Effect of Insecticides, Fungicides and Herbicides on Biofertilizer Bacteria and their Consortium

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ABSTRACT

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Combined soil application of Bio NPK and pesticides either simultaneous and sequential can enhanced plant growth parameters effectively and most of the pesticides tested *viz.* Profenofos 50% EC, Spinosad 45% SC, Dichlorvos 76% EC, Imidacloprid 30.5% EC, Fipronil 5% SC, Chlorpyrifos 20% EC, Pyraclotrobin 20% WG, Ridomil 68% WP, Quizalofop 5% EC and Finoxaprop 9% EC found safe to beneficial bacteria at the field recommended dose exhibiting no reduction of bacterial population compared with untreated control. However, broad spectrum and combination of fungicide (Mencozeb 63% + Carbendazim 12% WP) as well as multiple application of pesticide had inhibitory effect on tested individual bacterial members as well as Bio-NPK consortium. Thus, the studies indicated that all pesticides especially insecticides and herbicides are less detrimental to beneficial bacteria used as biofertilizers in common crop management practices.

Introduction

Micro-organism utilized to enhance availability of the major macronutrients, *viz.* nitrogen, phosphorus & potash and other micronutrients to the crops called Bio-fertilizer.

Biofertilizers are preparation containing active or latent cells of bacteria, fungi and cyanobacteria (blue green algae), which enhance grain and straw yields along with plant and soil health. Field experiments were

conducted to compare the efficiency of carrier based BGA bio-fertilizer (Khush *et al.*, 1992).

The effect of plant associated microorganism on plant growth and health; they enhance stress tolerance, provide disease resistance, aid nutrient availability and uptake and promote biodiversity (Satyendra *et al.*, 2010). They provide the plant with nutrient, resistance against disease and ability to combat worst climatic conditions (Alkhani *et al.*, 2006). Inoculation with a mixture of *Azotobacter* and *Azospirillum* with full doses

of rock phosphate and inorganic N-fertilizer, in combination with inoculation with vascular arbuscular mycorrhiza (VAM), improved growth of both datura (*Datura stramonium*) and ammi (*Ammi visnaga*: Fam. Umbelliferae) plants (Sharaf, 1995). The interaction among the rhizosphere, the root of higher plants and the soil borne microorganism has a significant role in plant growth & development. The organic compound, released by roots and bacteria play an important role in the uptake of mineral nutrient.

A pesticide is a substance or mixture of substances used for the management of the damage caused by pest.

The pests may be insects, bacteria, fungi, weeds, nematodes, or birds. When pesticides enter in to an organism via water or any other process it participate in the regular metabolism, by which a pesticides or chemical, changed into one or more different chemical (metabolite) within a living organism as a result metabolic product or metabolite, many become either more toxic than the original one.

Biofertilizers are progressively available in India market as one of the substitutes to reduce applications of costly chemical fertilizers in cultivated crops. Liquid biofertilizers enhancing the availability of macro nutrients *viz.* nitrogen (by fixing atmospheric N₂), phosphorus and potash (by solubilisation / mobilization from soil) and bacterial consortium (PGPR) formulations are more promising and updated technology (Vyas *et al.*, 2017) having several advantages over the conventional carrier based formulations.

In many studies, it is demonstrated that microorganisms are capable to grow in the

presence of noxious pesticides (Chandra *et al.*, 2016). A comprehensive study has been undertaken to investigate the effect of pesticides on five native agriculturally beneficial of liquid biofertilizers and Bio NPK, 'Anubhav' branded products of Anand Agricultural University very popular in farmers of western India for augmenting N, P and K in agricultural crops.

Materials and Methods

Pesticide used in the study

Seven insecticides, five fungicides and three herbicides commonly used in agricultural crop (as mentioned in table 1) are selected for compatibility with individual five bacteria of Bio-NPK and Bio-NPK consortium through agar plate assay and liquid tube assay in laboratory in this study.

Pesticide doses

All the pesticides were tested at three different doses *viz.* field recommended dose (X), half the field recommended dose (½X) and double the field recommended dose (2X). The Required test doses for the study were prepared in sterile distilled water and appropriate amount of pesticides pipetted in dilution tube under aseptic conditions.

The pesticide in granular/wettable powder form were added at 10 g of pesticide transferred to the 10 ml of sterile water and thoroughly mixed in sterile screw cap tubes and diluted to achieve desired doses just prior to experimentation.

Quantity of tested recommended dose (X)

$$= \frac{\text{Desired volume of water} \times \text{Recommended dose of pesticide}}{\text{Quantity of water}}$$

List of beneficial bacteria (Bio NPK) used in the study

Sr. No.	Organism	NCBI Accession No
1.	<i>Azotobacter chroococcum</i>	KF494187
2.	<i>Azospirillum lipoferum</i>	KR706179
3.	<i>Bacillus tequilensis</i>	JX403935
4.	<i>Bacillus coagulans</i>	KF933349
5.	<i>Bacillus licheniformis</i>	-

Agar well diffusion method

The agar plate surface was inoculated by spreading a volume of the microbial inoculum over the entire agar surface. A well with a diameter of 10 mm was punched aseptically with a sterile cork borer and a volume (20–100 µL) of the pesticide agent or extract solution at desired concentration was introduced into the well. Then, agar plates were incubated in incubator (time depending upon the test bacteria). The pesticides agent diffuses in the agar medium and inhibited growth of the microbial cultures and consortium formulation were tested. Zones or inhibition observed in the plates were considered as sensitive to the particular pesticide while the no inhibition shows the tolerance of bacteria towards particular tested pesticide. All the procedure repeated three times (Nene and Thapliyal, 1993).

Liquid assay

All the isolates were tested for pesticide tolerance in Nutrient broth with having (100 µl from the prepared doses) field recommended dose (X), half the field recommended dose (½X) and double the field recommended dose (2X) of pesticide keeping uninoculated control. 100 µl bacterial suspension of *A. chroococcum* (ABA-1), *A. lipoferum* (ASA-1), *B. tequilensis*, *B. coagulans* (PBA-16) and *B. licheniformis* individual and consortium product (5×10^8) CFU/ml was inoculated in nutrient broth tubes. Tubes were incubated at $28 \pm 2^\circ\text{C}$ for 3

to 5 days with control. Daily observations for bacterial growth (O.D.) were taken with the help of spectrophotometer at the time interval of 3, 4 and 5 days. The bacteria showing growth equal to the growth as in control was considered as compatible while the isolates having little or no growth was considered as sensitive to respective pesticide dose. All the procedure repeated three times (Abaidoo *et al.*, 2002).

Microbial count

Following serial dilution up to 10^{-7} , 0.1 ml of the respective dilutions were spreaded on respective solid media *i.e.* Jensen’s agar (for *Azotobacter*), N free BTB agar (for *Azospirillum*) and sperber (for PSB) and Aleksandrov’s media (for KMB) in laboratory studies (Tamizhazhagan *et al.*, 2016).

Results and Discussion

Results on compatibility of insecticides, fungicides and herbicides on individual bacterial members and Bio-NPK consortium in plate assay (Table 1 to 4 and Plate 1) revealed that growth of all the tested bacteria was not inhibited by the treatment of Spinosad 45% SC, Fipronil 5% SC and Imidacloprid 30.5% EC and Dichlorvos 76% EC at all three tested doses. Similar study was also conducted by Gnanchitra *et al.*, (2018) to access effect of Imidacloprid on the growth and multiplication rate of *Rhizobium* for a strategic eco-friendly seed treatment in pulse and they found that recommended dose of the

applied insecticide was not effecting survival and nodulation ability of the *Rhizobium* biofertilizer. Moreover, AAU Bio-NPK consortium found compatible with field recommended dose (X) and half recommended dose ($\frac{1}{2}X$) of all the tested pesticides. While growth of *A. chroococcum* was found to inhibited by double dose of Finoxaprop 9% EC, Mencozeb 63% + Carbendazim 12% WP and Quizalofop 5% EC, similarly for *A. lipoferum* growth inhibition was observed by double dose of Profenofos 50% EC and Mencozeb 63% + Carbendazim 12% WP for *B. licheniformis* growth inhibition was observed by double dose of Carbosulfan 25% EC, Chlorpyrifos 20% EC, Copper oxychloride 50% W/W, Mencozeb 63% + Carbendazim 12% WP, Propiconazole 25% EC, Ridomil 68% WP, Pyraclotrobin 20% WG, Pendimethalin 38.7% EC and Quizalofop 5% EC, for *B. tequilensis* growth inhibition was observed by double dose of Carbosulfan 25% EC, Chlorpyrifos 20% EC, Mencozeb 63% + Carbendazim 12% WP, Pyraclotrobin 20% WG, Quizalofop 5% EC and Finoxaprop 9% EC, similarly growth inhibition was observed for Bio-NPK consortium by Copper oxychloride 50% W/W, Mencozeb 63% + Carbendazim 12% WP, Ridomil 68% WP, Quizalofop 5% EC and Finoxaprop 9% EC. Growth inhibition of *A. chroococcum*, *A. lipoferum* and *B. coagulans* was observed for recommended dose (X) of Mencozeb 63% + Carbendazim 12% WP only, similarly for *B. licheniformis* growth inhibition was observed by Mencozeb 63% + Carbendazim 12% WP (Plate 2) and Propiconazole 25% EC, *B. tequilensis* growth inhibition was observed by Carbosulfan 25% EC and Mencozeb 63% + Carbendazim 12% WP. Half dose ($\frac{1}{2}X$) of fungicide Mencozeb 63% + Carbendazim 12% WP found to inhibit growth of all the tested individually bacteria. Interestingly, Sethi *et al.*, (2013) studied effects of synthetic pyrethroids on phosphate solubilizing activity of microorganisms.

During their study, they found that, amongst the four synthetic pyrethroid insecticides studied, all of them showed remarkable effect on the populations of P-solubilizing microorganisms. The effect of Deltamethrin on *Bacillus*, *Rhizobium* and *Pseudomonas* was more pronounced as compared to Alpha-Cyhalothrin, Fenvelerate and Cypermethrin. The application of Cypermethrin inhibited the activity of *Pseudomonas* and *Serratia*, significantly as compared to Fenvalerate, Alpha-Cyhalothrin and Deltamethrin. The inhibitory effect of Fenvelerate was more pronounced in case of *Pseudomonas*, *Azotobacter* and *Rhizobium* as compared to other three insecticides. Lastly alpha-Cyhalothrin did not show any remarkable inhibitory effect on Phospahte solubilizing activity as compared to remaining three insecticides.

Results of the liquid assay revealed that different doses of profenofos, spinosad, dichlorvos, imidacloprid and fipronil were found compatible with all the individual bacterial members and Bio-NPK consortium at all three test doses. While, double dose (2X) of Carbosulfan 25% EC, Copper oxychloride 50% W/W, Pendimethalin 38.7% EC, Propiconazole 25% EC, Ridomil 68% WP were not found compatible with *B. licheniformis*. Similarly, double dose of Chlorpyrifos 20% EC and Pyraclotrobin 20% WG were not found compatible for *B. tequilensis* and *B. licheniformis*. Moreover, Quizalofop 5% EC was not found compatible for *A. chroococcum*, *B. tequilensis* and *B. licheniformis* at double dose (2X). Finoxaprop 9% EC was not found compatible for *A. chroococcum*, *B. licheniformis* and *B. coagulans* at double dose. Same way, Mencozeb 63% + Carbendazim 12% WP was not found compatible with *A. chroococcum*, *A. lipoferum*, *B. tequilensis*, *B. coagulans* and *B. licheniformis* and Bio-NPK consortium at double and recommended doses. While, A.

chroococcum, *A. lipoferum*, *B. tequilensis*, *B. coagulans* and *B. licheniformis* and Bio-NPK consortium were found compatible with half dose of Mencozeb 63% + Carbendazim 12% WP. As shown in Figure 1 the liquid assay of Pendimethalin 38.7% EC at three different field recommended doses *i.e.* ½X, X and 2X, it was found that growth was continuously increased up to 5 days and on 5th day growth was found to be maximum. Growth of *B. licheniformis* is decreased at double doses at 3, 4 and 5 days significantly. At 5th day population density of *B. licheniformis* was (1.17) as compare to bacterial inoculated control (1.97). The growth of organisms continuously increased day to day. Pendimethalin 38.7% EC proved compatible for all the organisms. Moreover, Quizalofop 5% EC, continuous increase of bacterial growth up to 5 days was reported and on 5th day population density of the broth was found to be maximum. At 5th day density of *A. chroococcum*, *B. tequilensis* and *B. licheniformis* was (1.50), (1.52) and (1.22) as compare to bacterial inoculated control (2.26), (2.26) and (1.97) respectively and found sensitive to the double dose of herbicide. At 3, 4 and 5 days the growth of organism found affected by the double dose of herbicide. Quizalofop 5% EC is not compatible with *A. chroococcum*, *B. tequilensis* and *B. licheniformis* at double dose only. Hence, other organism *A. lipoferum*, *B. coagulans* and Bio-NPK consortium found compatible

for any dose for pest management practices. Similarly for Finoxaprop 9% EC, it was noticed that continuous increase in bacterial growth up to 5 days and on 5th day growth was found to be maximum. At 5th day population density of *A. chroococcum*, *B. coagulans* and *B. licheniformis* was (1.60), (1.56) and (1.12) as compare to bacterial inoculated control (2.26), (2.50) and (1.97) respectively and found sensitive to the double dose of herbicide. Growth of *A. chroococcum*, *B. coagulans* and *B. licheniformis* found sensitive at double dose at time interval. Thus, Finoxaprop 9% EC is not compatible with *A. chroococcum*, *B. coagulans* and *B. licheniformis* at double dose.

Gaherval *et al.*, (2015) studied the impact of chemical (2, 4-D, dimethoate and carbendazim) and herbal product by well diffusion, disk diffusion and MIC methods, on nitrogen fixers against *Rhizobium*, *Azotobacter* and *Azospirillum* and found that growth inhibition against all three bacteria was different and veering. Carbendazim showed the zone of inhibition against *Azospirillum* and *Rhizobium* bacteria but did not show inhibition against *Azotobacter*. Laboratory evaluation indicated that amongst pesticides, insecticides and herbicides are less detrimental to beneficial bacteria useful as biofertilizers in cotton crop practices at recommended dose.

Table.1 Pesticides used in the study

Sr. No.	Insecticides	Fungicides	Herbicides
1	Profenofos 50% EC	Copper oxychloride 50% W/W	Pendimethalin 38.7% EC
2	Spinosad 45% SC	Mencozeb 63% + Carbendazim 12% WP	Quizalofop 5% EC
3	Fipronil 5% SC	Propiconazole 25% EC	Finoxaprop 9% EC
4	Imidacloprid 30.5% EC	Ridomil 68% WP	
5	Dichlorvos 76% EC	Pyraclotrobin 20% WG	
6	Carbosulfan 25% EC		
7	Chlorpyrifos 20% EC		

Table.2 Effect of different pesticides on individual bacterial member as well as Bio-NPK consortium

Treatment	Zone of inhibition (mm)														
	Profenofos 50% EC			Spinosad 45% SC			Fipronil 5% SC			Imidacloprid 30.5% EC			Dichlorvos 76% EC		
	Dose			Dose			Dose			Dose			Dose		
	½X	X	2X	½X	X	2X	½X	X	2X	½X	X	2X	½X	X	2X
<i>A. chroococcum</i>	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
<i>A. lipoferum</i>	R	R	5	R	R	R	R	R	R	R	R	R	R	R	R
<i>B. licheniformis</i>	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
<i>B. tequilensis</i>	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
<i>B. coagulans</i>	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Bio-NPK	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Control	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R

Note: R stands for resistant (0 mm)

Table.3 Effect of different pesticides on individual bacterial member as well as Bio-NPK consortium

Treatment	Zone of inhibition (mm)														
	Carbosulfan 25% EC			Chlorpyrifos 20% EC			Copper oxychloride 50% W/W			Mancozeb 63% + Carbendazim 12% WP			Propiconazole 25% EC		
	Dose			Dose			Dose			Dose			Dose		
	½X	X	2X	½X	X	2X	½X	X	2X	½X	X	2X	½X	X	2X
<i>A. chroococcum</i>	R	R	R	R	R	R	R	R	R	4	5	7	R	R	R
<i>A. lipoferum</i>	R	R	R	R	R	R	R	R	R	3	5	7	R	R	R
<i>B. licheniformis</i>	R	R	15	R	R	9	R	R	9	5	8	10	R	5	9
<i>B. tequilensis</i>	R	8	10	R	R	14	R	R	R	4	6	8	R	R	R
<i>B. coagulans</i>	R	R	R	R	R	R	R	R	R	3	5	6	R	R	R
Bio-NPK	R	R	R	R	R	R	R	R	5	R	R	8	R	R	R
Control	R	R	R	R	R	R	R	R	R	R	R	0	R	R	R

Note: R stands for resistant (0 mm)

Table.4 Effect of different pesticides on individual bacterial member as well as Bio-NPK consortium

Treatment	Zone of inhibition (mm)														
	Ridomil 68% WP			Pyraclostrobin 20% WG			Pendimethalin 38.7% EC			Quizalofop 5% EC			Finoxaprop 9% EC		
	Dose			Dose			Dose			Dose			Dose		
	½X	X	2X	½X	X	2X	½X	X	2X	½X	X	2X	½X	X	2X
<i>A. chroococcum</i>	R	R	R	R	R	R	R	R	R	R	R	11	R	R	10
<i>A. lipoferum</i>	R	R	R	R	R	R	R	R	R	R	R	0	R	R	0
<i>B. licheniformis</i>	R	R	8	R	R	9	R	R	14	R	R	9	R	R	0
<i>B. tequilensis</i>	R	R	R	R	R	R	R	R	R	R	R	7	R	R	0
<i>B. coagulans</i>	R	R	R	R	R	R	R	R	R	R	R	5	R	R	7
Bio-NPK	R	R	6	R	R	R	R	R	R	R	R	6	R	R	6
Control	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R

Note: R stands for resistant (0 mm)

Fig.1 Effect of Pendimethalin 38.7% EC on individual bacteria and Bio-NPK consortium by liquid assay

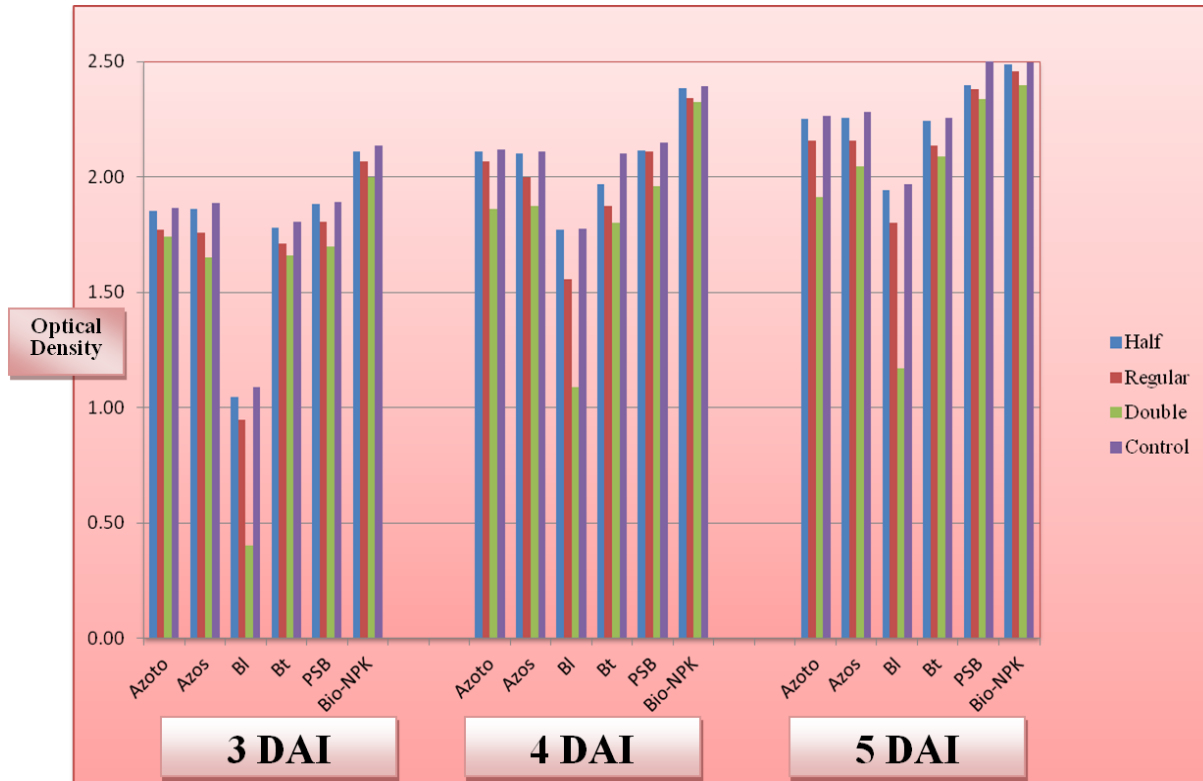


Plate.1 Compatibility of Bio-NPK consortium at field recommended dose with A) Finoxaprop 9% EC, B) Dichlorvos 76% EC, C) Carbosulfan 25% EC, D) Pendimethalin 38.7% EC, E) Spinosad 45% SC and F) Propiconazole 25% EC by plate bio assay

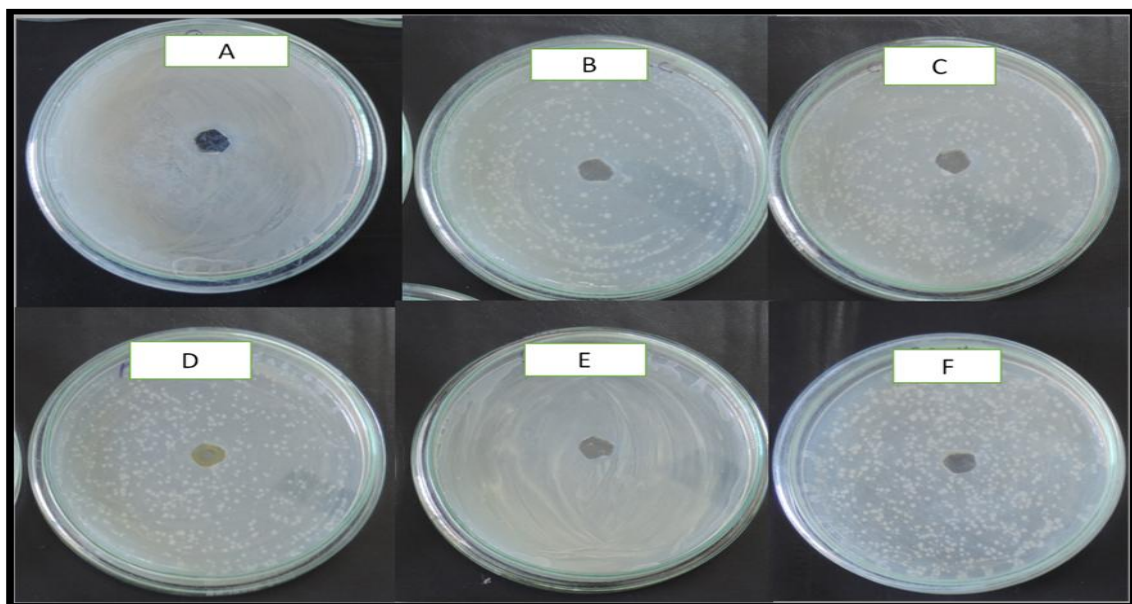
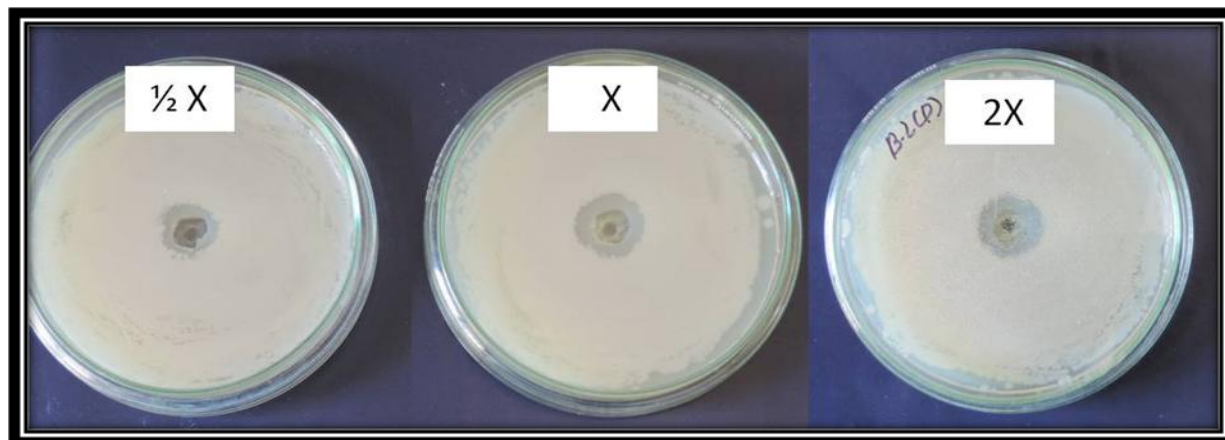


Plate.2 Compatibility of different doses of Mancozeb 63% + Carbendazim 12% WP with *B. licheniformis*



However, indiscriminate use of pesticides on crop like double the field recommended dose application can exert some harmful effect, but broad spectrum and combination of fungicide as well as multiple application of pesticide are detrimental to tested individual bacterial members as well as Bio-NPK consortium. Thus all the pesticides are not harmful to beneficial bacteria used in crop management practices.

In conclusion, different pesticides at their recommended doses found compatible with representative soil beneficial microorganisms. But, higher concentration (dose) in comparison to recommended doses found to act negatively on soil beneficial microorganisms. Interestingly soil applied insecticides and herbicides commonly used in crop management practices are recorded with less detrimental to beneficial bacteria in compare to fungicides, particularly combination of new fungicide molecules.

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