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Research Article

LABORATORY STUDIES ON THE EFFECT OF SOME PLANT PROTECTION CHEMICALS AGAINST *BEAUVERIA BASSIANA*

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Abstract: Present agricultural practices encourage the use of two or more than two chemicals to achieve broad spectrum plant protection effect. Not so much is known concerning the effect of these chemicals applied singly or in combination, on the ecology of micro flora, microbe population, including pathogenic fungus and also the antagonistic fungi use of these chemicals may prove beneficial not only in weed control and insect control but also as a means of controlling certain soil borne fungi through their effect in non-target organisms.

Keywords: *Beauveria bassiana, Plant protection chemicals, Poisoned food technique*

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Introduction

Usually farmers in India are unaware of biological control agent and their possible application in the field of controlling pests in agriculture. They have been using many synthetic pesticides such as DDT, BHC, Endosulphan, Demerom, Monocrotophos, Malathion etc. for controlling pests. Evidence of chemical pesticides poisoning in human and wild life contamination of ecosystem, high level of pesticides in food articles are becoming increasingly of great concern. The export of agricultural commodities to developed countries has also suffered due to chemical pesticides application. A large number of pesticides used are poisoning the nature, men and other warm-blooded animals and also leaves residue of pesticide. The efficiency of these methods largely depends upon crop and their varieties particularly leaf, stem fruits, climatic condition such as temperature and rainfall, pH of soil, texture of soil. The ill effect of chemical pesticides on human health and the environment, development of resistant in pest to pesticides higher level of pesticides residue in food item. There is carrying need to develop suitable alternative to chemical pesticides for use in pest control. Biological control methods are one such alternative which provide non-hazardous approach to pest control. Several species of bacteria virus fungi are being exposed on biological agents of pests. Biological control has been broadly defined as encouragement of beneficial organism already existing in locality or of the introduction of suitable new species of exotic parasite organism which are parasitic on harmful insect in the locality where pest is thriving with a view to control pests. Fungal disease insect which have been recorded in nineteen century and may have been caused by fungal belonging to Phycomycetes, Ascomycetes, Basidiomycetes and fungi imperfecti have been mostly considered for insect suppression. Genera Beauveria metarrhizium of fungi and imperfect, ectomophora and coelomyces of phycomycetes, cardyceps of ascomycetes have received more attention. Attempts have been made to reduce insect population through introduction of fungi parasitic on insect. Successful experiment was conducted in America for control of chinch bug (*Blesstus luclptorous*) by white muscardine fungus *Beauveria bassiana*. Fungal spore is dispersed in water and sprayed on plants when they come in contact within the body later, they kill the insect. The insect either producing the disease or interfering the normal function of insect through mycilial net. Sosnowska 2013 used automopathogenic fungus *B. bassiana* for control of *Leptinotorsa decunlineata* [1]. Van Lenteren *et al*. 2017 reported *B. bassiana* in controlling the brown plant hopper [2]. *Beauveria bassiana* mycelium i.e., white or lightly coloured with a white fluffy to powdery appearance conidiophore single irregularly grouped or inverticillate dustors, conidi a hylaine, rainded avoid T called borne singly on small storigmata, parasitous insect. Biological control of pest by micro-organism has been known all over the world of but interest and increased effort in this area has gained momentum in last decade due to shift in opinion that this method can be combined in integrated pest management (IPM). IPM in management of soil borne insect pests should aim at combining all principles of pest management including bio-control agent. Among the various autogonistic micro-organism used as bio-control agent against pest control, white inuscardine fungus *Beauveria bassiana* is well known for its potential as on effective bio-pesticide. Therefore, there is urgent need to study the compatibility of this fungus with commonly used Agro-chemicals pesticides before recommending its application against the pest.

Material and Methods

The studies entitled "Laboratories studies on the effect of some plant protection chemicals against *Beauveria bassiana*" was conducted in Research Laboratory, Department of Plant Pathology & Nematology, Sam Higginbottom University of Agriculture, Technology and Sciences, Naini, Prayagraj, 211007, Uttar Pradesh. Cleaning and sterilization of glass ware used in the experiment were thoroughly washed with the help of detergent powder and tap water and then dried. The petri dishes and pipettes were wrapped in clean paper and sterilized in an oven at temperature of 160-180°C for four hours. For isolation and growing of fungus Sabouraud Dextrose Agar (SDA) medium was used. The fungus was isolate from dead interbella quadrinotata, the guava bark coding caterpillar larva on guava tress in orchard of Sam Higginbottom University of Agriculture, Technology and Sciences, Naini, Prayagraj, 211007, Uttar Pradesh. Collected this culture was then purified on SDA medium and maintained for used in various experiment.

Table-1 Effect of 2-4D on the growth of Beauveria bassiana on SDA medium on 7th days after inoculation

Treatment	Concentration (ppm)	Replication				Total	Mean
		1	2	3	4		
T_O	0	2.35	2.6	2.3	2.2	9.45	2.36
T_1	1	2.22	2.5	2.3	2.12	9.14	2.28
T_2	10	2.65	2.4	2.4	2.15	9.6	2.4
T_3	100	2.1	2.2	2.5	2	8.8	2.2
T4	1000	1.1	1	0.95	0.9	3.95	0.98

Table-2 Effect of 2-4D on the growth of Beauveria bassiana on SD broth medium on 15 days after inoculation

Treatment	Concentration (ppm)	Replication				Total	Mean
		1	2	3	4		
T_O	0	3.36	3.43	2.7	2.8	12.29	3.07
T_1	1	2.62	2.69	2.85	2.75	10.91	2.72
T_2	10	3.84	3.24	2.88	3.02	12.98	3.2
T_3	100	3.46	3.33	2.46	2.55	11.8	2.95
T4	1000	1.82	2.95	1.59	1.75	8.11	2.02

Table-3 Effect of Butachlor on the growth of Beauveria bassiana on SDA medium on 7th days after inoculation

Treatment	Concentration (ppm)	Replication				Total	Mean
		1	2	3	4		
T_O	0	3	2.2	3.55	2.3	11.05	2.76
T_1	1	3.2	2.25	2.3	2.85	9.6	2.4
T_2	10	3	2.5	2.2	2.1	9.8	2.45
T_3	100	2.15	2.28	2.1	2.65	9.18	2.29
T4	1000	1.55	2	2.6	2.9	9.05	2.26

Table-4 Effect of Butachlor on the growth of Beauveria bassiana on SDA medium on 15 days after inoculation

Treatment	Concentration (ppm)	Replication				Total	Mean
		1	2	3	4		
T_O	0	2.03	2.69	2.69	2.6	10.01	2.5
T_1	1	1.96	2.76	2.86	2.25	9083	2.45
T_2	10	2.78	3.38	2.95	2.45	11.56	2.89
T_3	100	2.68	2.05	2.79	2.62	10.14	2.52
T4	1000	2.02	1.92	1.77	2	7.71	1.92

Table-5 Effect of Captan on the growth of Beauveria bassiana on SD broth medium on 7th day after inoculation

Treatment	Concentration (ppm)	Replication				Total	Mean
		1	2	3	4		
T_O	0	3	2.45	2.4	2.3	10.15	2.53
T_1	1	2.43	1.9	2.2.	2.5	9.03	2.25
T_2	10	2.45	2.6	2.15	2.45	9065	2.41
T_3	100	2.3	2.6	2.8	1.6	9.3	2.32
T4	1000	1.1	1.3	1.1	0.9	4.3	1.02

Table-6 Effect of Captan on the growth of Beauveria bassiana on SD broth medium on 15 days after inoculation

Treatment	Concentration (ppm)	Replication				Total	Mean
		1	2	3	4		
TO	0	4.37	4.34	4.48	4.07	17.36	4.3
T1	1	3.63	3.82	3.7	3.69	14.84	3.7
T2	10	2.29	2.93	2.53	2.61	10.36	2.6
T3	100	2.81	3.02	2.78	2.81	11.42	2.8
T4	1000	1.96	1.03	1.09	1.05	5.1	1.3

Table-7 Effect of vitavex on the growth of Beauveria bassiana on SDA medium on 7th days after inoculation

Treatment	Concentration (ppm)	Replication				Total	Mean
		1	2	3	4		
T_O	0	2.8	1.9	2	2.5	9.25	2.31
T_1	1	1.7	1.8	1.65	2	7.15	1.78
T_2	10	1.65	1.75	1.65	1.8	6.85	1.71
T_3	100	1.5	1.65	1.55	1.7	6.4	1.6
T4	1000	0.8	1.1	1	0.9	3.8	0.95

Table-8 Effect of vitavex on the growth of Beauveria bassiana on SD broth medium on 15 days after inoculation

Treatment	Concentration (ppm)	Replication				Total	Mean
		1	2	3	4		
T_O	0	2.83	3.19	3.17	3.24	12.43	3.1
T_1	1	3.37	3.13	2.95	2.87	12.32	3.08
T_2	10	2.99	3.13	2.82	3.02	11.96	2.99
T_3	100	2.97	3.04	2.66	3.02	11.69	2.92
T4	1000	0.8	1	1.1	0.8	3.7	0.92

Table-9 Effect of phorate on the growth of Beauveria bassiana on SDA medium on 7th days after inoculation

Treatment	Concentration (ppm)	Replication				Total	Mean
		1	2	3	4		
T_O	0	2.2	2.2	2.1	2.25	8.55	2.1
T_1	1	2.25	1.85	1.5	2	7.6	1.9
T_2	10	2	2.5	1.4	1.65	7.55	1.9
T_3	100	1.9	2.3	1.35	1.4	6.95	1.7
T4	1000	1.25	1.15	1.2	1.18	4.78	1.2

Table-10 Effect of phorate on the growth of Beauveria bassiana on SD broth medium on 15 days after inoculation

Treatment	Concentration (ppm)	Replication				Total	Mean
		1	2	3	4		
T_O	0	2.21	2.25	2.6	2.21	9.27	2.3
T_1	1	1.74	1.83	2.25	1.82	7.64	1.9
T_2	10	1.69	1.65	2.01	1.72	7.07	1.7
T_3	100	1.81	1.74	1.64	1.69	6.88	1.7
T4	1000	81	0.9	1.01	0.98	3.7	0.92

Table-11 Effect of carbofuran on the growth of Beauveria bassiana on SDA medium on 7 days after inoculation

Treatment	Concentration (ppm)	Replication				Total	Mean
		1	2	3	4		
T_O	0	2.83	1.04	2.67	2.54	9.08	2.3
T_1	1	1.6	1.05	2.7	3.73	9.08	2.3
T_2	10	2.46	1.89	1.88	2.01	8.24	2.1
T_3	100	2.66	2.46	1.11	1.82	8.01	2
T4	1000	1.1	1.4	1.4	1.2	5.1	1.3

Table-12 Effect of carbofuran on the growth of Beauveria bassiana on SD broth medium on 15 days after inoculation

Treatment	Concentration (ppm)	Replication				Total	Mean
		1	2	3	4		
T_O	0	2.33	3.16	2.99	2.55	11.03	2.7
T_1	1	2	2.95	1.59	2.12	8.66	2.1
T_2	10	3.14	2.57	2.28	2.14	10.13	2.5
T_3	100	2.58	3.46	3.38	3.13	12.73	3.2
T4	1000	4.04	3.32	2.89	2.83	13.08	3.33

Table-13 Effect of malathion on the growth of Beauveria bassiana on SDA medium on 7 days after inoculation

Treatment	Concentration (ppm)	Replication				Total	Mean
		1	2	3	4		
T_O	0	2.55	2.3	2.9	2.3	9.75	2.4
T_1	1	2	2.85	2.65	2.2	9.7	2.4
T_2	10	1.5	1.95	1.75	2	7.2	1.8
T_3	100	1.4	1.75	1.15	2	6.3	1.6
T4	1000	1.1	1.1	0.95	0.9	3.95	0.98

Table-14 Effect of malathion on the growth of Beauveria bassiana on SD broth medium on 15 days after inoculation

Treatment	Concentration (ppm)	Replication				Total	Mean
		1	2	3	4		
T_O	0	2.42	2.3	2.9	2.21	8.64	2.2
T_1	1	2.4	2.8	2.4	2.15	9.75	2.4
T_2	10	2.08	2.3	2	2.1	9.48	2.1
T_3	100	2.7	2.56	2.4	1.95	9.6	2.4
T4	1000	0.6	0.13	0.11	0.15	0.99	0.24

Table-15 Effect of endosulphan on the growth of Beauveria bassiana on SDA medium on 7 days after inoculation

Treatment	Concentration (ppm)	Replication				Total	Mean
		1	2	3	4		
T_O	0	2.62	2.15	2.5	2.25	9.52	2.4
T_1	1	1.8	1.6	1.65	2	7.05	1.8
T_2	10	1.5	1.35	1.6	1.9	6.35	1.6
T_3	100	1.1	1.4	1.4	1.2	5.1	1.3
T4	1000	0.9	1.1	0.55	0.6	3.05	0.8

Table-16 Effect of endosulphan on the growth of Beauveria bassiana on SDA medium on 15 days after inoculation

Treatment	Concentration (ppm)	Replication				Total	Mean
		1	2	3	4		
T_O	0	2.5	2.8	2.42	2.36	10.08	2.5
T_1	1	2.25	2.73	2.2	2.2	9.058	2.2
T_2	10	2.04	2.42	2.12	2.52	9.1	2.3
T_3	100	2.4	2.48	2.46	2.2	9.54	2.4
T4	1000	0.8	1.1	1.1	1.2	4.1	1

The culture of *Beauveria bassiana* was purified from isolation dishes and maintained by periodic sub culturing in SDA slants at every 15 days. The required dilution of chemicals (1, 10, 100, 1000 ppm) were prepared taking an active ingredient into consideration. The various prepared pesticides were tested against *B. bassiana* by poison food technique [3]. The principle involved in this technique is to poison the nutrient medium with fungi-toxicants and then allow the test fungus to grow on such medium. In this technique both solid and broth media can be used. With the help of sterilized cark borer disc of 0.5 cm diameter were cut from actively growing culture of *B. bassiana* and placed in the centre of each disc. Four replications for each treatment including control were maintained. Inoculated dishes were incubated at 28-29°C and observation on colony diameter (in cm) were recorded at 7th days after inoculation. In broth medium, agar discs of test fungus were inoculated in conical flask. Four replicates for each treatment including control were maintained. The inoculated flask was incubated at 28-29°C for 15 days liquid out.

Result and Discussion

The Present laboratories study was conducted to study on the effect of some plant protection chemicals on growth of entomo-pathogenic fungus *Beauveria bassiana*. The fungus was isolated from dead Inderbella larvae on Guava (*Psidium guajava*) trees in orchard of Allahabad Agricultural Institute, Allahabad.

Effect of herbicides on *Beauveria bassiana*

Effect of two herbicide *viz*., 2-4 D and Butacholar was studied on growth of *Beauveria bassiana* on SDA medium on seven days and on SD broth medium on 15 days respectively. 2-4 D on SDA medium significantly inhibited the growth of fungus of all concentration compared to control on 7th day. The growth in T2 and T3 treatment was not different from each other but was more than T4. On SD broth medium on 15th day mycelium weight was significantly more in treatment T4, T1, T3 and T0 compared to T2. Effect of butachlor on SDA medium shows that the radial growth of fungus was not significantly different between the treatment and was in the order TO < T2 < T3 < T4 on 7th day. Effect of butachlor on 15 days mycelium weight was significantly more in treatment T3, T0, T1, T4 compared to T2. Treatment T0, T1, T2, T3 were not significantly different from each other. These two herbicides used in present studies namely 2-4 D and Butachlor, generally inhibited the growth in both the medium. There was in general a direct relationship between increase in concentration of chemical and inhibition of fungal growth [4]. Similar results were also obtained by Majchrowicz *et al.* (1993) and Rashid *et al* (2010) [5,6].

Effect of fungicides on *Beauveria bassiana*

Effect of two fungicide *viz*., captan and vitavex was studied on the growth of *Beauveria bassiana*

Effect of captan on SDA medium showed that radial growth of fungus was significantly more in treatment T1, T2, T3 and T4 compared to T0. On SD broth medium on 15 days, mycelial weight was significantly more in treatment T4, T3, T2 and T1 compared to T0. Treatment T2 and T3 were not differently to each other. Effect of vitavex on SDA medium showed that the radial growth of fungus was significantly different in all treatment was in the order T0 < T1 < T2 < T3 < T4. On SD broth medium on 15 days mycelium weight was more in treatment T4, T3, T2 and T1 compared to TO. Treatment T2 and T3 were not differently to each other. All the two fungicides used in the present studies generally inhibited the growth of *Beauveria bassiana* at all four concentration. There was also seen in general, a direct relationship between increase in chemical concentration and inhibition in the fungus growth. Inhibition in growth of several fungi with Bavistin has been reported by Patil (2006) and Rashid *et al* (2010) [7].

Effect of nematicide on *Beauveria bassiana*

Effect of two nematicide *viz*., phorate and carbafuran was studied on the growth of *Beauveria bassiana*

Effect of phorate on SDA medium shows that the radial growth of fungus was significantly different in all treatment was in the order TO < T1 < T2 < T3 and T4. Effect of phorate on Broth medium on 15 days mycelium weight was significantly more in treatment T0 < T1 < T2 = T3 < T4 compared to T0. Effect of carbofuran on SDA medium showed that the radial growth of fungus was significantly more in treatment T1, T2, T3 and T0= T1 on 7 th day. On 15 days mycelium weight was significantly more in treatment T1< T2 < T0 < T3=T4. Phorate and carbafuran a granular nematicide used in present studies inhibited the growth of *Beauveria bassiana*. Inhibitory effect of several nematicide has been reported by Faria *et al*. (2007), Majchrowicz *et al*. (1993) and Tkaczuk *et al*. (2005) [8].

Effect of insecticides on *Beauveria bassiana*

Effect of two insecticides *viz*., malathione and carbafuran was studied on growth of *Beauveria bassiana* on SDA medium on 7th day and SD broth medium on 15 day respectively. Effect of malathion on SDA medium shows that the radial growth of fungus was significantly more in treatment T0 = T1 < T2 < T3 < T4. On SD broth medium on 15 days mycelium weight was significantly more in treatment T3 < T4 < T1 < T0 < T2. Effect of endosulphan on SDA medium showed that the radial growth of fungus was significant in T0 < T1 < T2 < T3 < T4 on 7th day. Effect of endosulphan on SD broth medium on 15 days mycelial weight was non-significant. Difference between the treatment and was in the order T0 < T2 < T3 < T1 < T4. Malathian and Endosulphan in present studies generally inhibited the growth of *Beauveria bassiana* at all the four concentration on both medium. Similar reports were cited by Pelizza *et al*. (2013) and Sosnowska (2013) and Tkaczuk *et al*. (2012) [9,10].

Application of research: Study the compatibility of this fungus with commonly used Agro-chemicals pesticides

Research Category: Agriculture Chemistry

Abbreviations: SDA: Sabouraud Dextrose Agar

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