

DEVELOPMENT OF LIQUID FORMULATION FOR THE DUAL PURPOSE OF CROP PROTECTION AND PRODUCTION

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ABSTRACT

Intensive commercial farming involves excessive use of chemical fertilizers and pesticides. It is feared that practice of using chemical fertilizers and pesticides continually would result in gradual aggravation of soil fertility. Use of agriculturally important microorganisms in different combinations is the only solution for restoration of soils. A bio formulation using humic acid and a suitable microorganism namely, *Pseudomonas fluorescens* has been developed to replace chemical fertilizers. This liquid formulation in addition to facilitates long shelf life, zero contamination, no need of carriers, convenience of handling, storage and transportation has easy to use with irrigation. The mixed formulation of humic acid along with the microorganism namely *Pseudomonas fluorescens* can be used for the dual purpose viz., crop protection and enhanced production. The liquid bio-formulation was tested and compared for viability as well as its inhibitory characteristics against *Fusarium oxysporum*, a fungus which cause wilt of tomato. Field studies were conducted for two crop varieties- radish and tomato. Cell viability tests were carried out for the bio-formulations by plate count method. The results revealed that both *in vivo* and *in vitro* the formulation containing humic acid showed better support for the viable cells as well as leafing characteristic in pot kept in field as compared to the control. The formulation was able to inhibit the wilt of tomato caused by *Fusarium* thus confirming its protective property.

Key Words : Liquid formulation, Crop production, Protection, Humic acid, Microorganism

INTRODUCTION

Improvement of the crop production and the protection of the crop from insects and pests attack are the major goals of the scientists. Various types of methodologies ranging from fertilizers, manures, organic farming, crop rotation, genetic improvement are employed for the improvement of production of crops. Various chemical pesticides, insecticides and natural ways of protection of crops are employed for the crop protection. A great deal of attempt is made in the scientific community to improve the production and protection of the crop. Liquid Bio-formulations are the microbial preparations containing specific beneficial microorganisms which are capable of fixing or solubilizing or mobilizing plant nutrients

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by their biological activities. This work concentrates on the development of a liquid formulation which will enhance the production of the crop and protect it from the chemical pesticide and the insecticide effects. The application of chemical pesticides and fertilizers leads to decrease the soil fertility, which can be restored by the application of different combinations of microorganisms that help in improving the soil nutrient.¹ Humic acid in combination with *Pseudomonas fluorescens* can serve the dual purpose of production and protection for the crops. Humic acid, a derivative of Lignite coal² can be a suitable fertilizer for the soil while *Pseudomonas fluorescens*, which acts as a microbial pesticide. Beneficial micro-organisms as bio-control agents can improve plant growth by enhancing the resistance mechanism of the

plants and increase the product yield of crops and cultivars.³

Background

The bioformulations, which have molecular weight in the range of 5000-30000 and are classified into two categories as humic acid and fulvic acid. They are the complex mixtures of many different acids containing carboxyl and phenolate groups so that the mixture behaves functionally as a dibasic acid or occasionally, as a tribasic acid.⁴ These molecules act as bio-stimulants and chelating agents provide stimulus for the growth of plants and maturation of seedlings and make unavailable trace quantities of minerals available for plants. They act as a food source for beneficial soil microorganisms, also prevent the soil erosion and in turn enhance the water holding capability of soil.^{5,6} Humic acid is not a fertilizer as it does not directly provide nutrients to plants but is a compliment to fertilizer.⁷ The activities of beneficial soil microbes are crucial for the sustainability of any soil and plant growth. Humic acid stimulates microbial activity by providing the indigenous microbes with a carbon source for

food, thus encouraging their growth and activity. Soil microbes are responsible for solubilizing vital nutrients such as phosphorus which is absorbed by the humic acid and in turn made available to the plant.^{8,9} The microbes are also responsible for the continued development of humus in the soil by breaking down or decomposing the organic matter. Biological control agent is the use of PGPR or plant growth promoting bacteria and their derivatives for the control of plant diseases.¹⁰⁻¹⁶

MATERIAL AND METHODS

Preparation of liquid formulation

A liquid bio-formulation containing a mixture of 2% (3g) humic acid and *Pseudomonas fluorescense* as biocontrol agent was prepared and stored in the refrigerator. This liquid formulation was poured in 3 test flasks containing 2% (3 gram) humic acid, Kings B broth and 3 control flasks. The control flasks contains the Kings B broth and without humic acid. The pH was maintained at 7. The *Pseudomonas fluorescens* was cultured and sub-cultured in Kings B Agar medium (**Fig. 1** and **Fig. 2**).



(A)



(B)

Fig. 1 : Slants containing sub-cultured *Pseudomonas fluorescense*



Fig. 2 : Liquid formulation- test and control

Lab test

The formulation was tested for its efficiency in inhibiting the growth of the fungus *Fusarium* that causes wilt of tomato. The test flasks were inoculated aseptically with *Pseudomonas fluorescens* using laminar air flow and incubated in orbital shaker at 120 rpm at 30°C for 48 hours. Inoculated broth were transferred into centrifuge tubes and centrifuged at 10,000 rpm at 4°C for 10 minutes. The pellet was re-suspended in 10 millimolar phosphate buffer containing 1% glycerol. Viable cell count was determined by serial dilution and plate count method.

The *Fusarium oxysporum* obtained from Microbial Type Culture Collection (MTCC), Chandigarh. The culture was sub-cultured and maintained on sterilized petri plates containing 120 ml of Potato Dextrose Agar (PDA) Medium. 20ml of the media was poured into separate sterilized petriplates and inoculated with *Fusarium*. Two sets of triplicates were maintained: i) *Fusarium* and PDA medium, ii) *Fusarium*, PDA medium and 1ml of the bio-formulations and incubated at 30°C for 72 hours.

Field test

The bio-formulation was tested on two crops viz., the radish and tomato by pot culture studies. The growth characteristics of these two plants can be easily studied as they germinate within 3-7 days and mature in a short span of time. The morphological characteristics such as the number of leaves produced among the controls and experimental plants were observed and compared on a weekly basis. The length of the stem and number of leaves produced were recorded for the tomato plant, while radish was studied for the number of leaves produced and compared with the control. Since, the tuber is positively geotrophic, the tuber was not disturbed for the purpose of recording.

Both tomato and radish plants were potted and maintained in the following manner: One set containing the seeds mixed with *Fusarium* and another set containing the seeds *Fusarium* and the liquid bio-formulation. The radial growth

was measured after 6-7 days followed by its test on pot cultures. The growth of the plants was observed and recorded. Field studies were conducted to test the protective property of the prepared liquid bio-formulation on two sets of tomato and potato plants. The experimental plan was made in the following manner :

1. Control set containing tomato seeds + fungal pathogen *Fusarium oxysporum lycopersici*.
2. Experimental set containing tomato seeds + fungal pathogen + liquid bio-formulation.

Similar pattern was followed in radish also and all the sets were monitored at regular intervals.

RESULTS AND DISCUSSION**Lab test**

Using the liquid bio-formulation the cell viability tests were carried out by plate count method every fortnight. Plates were compared with a control liquid formulation. The cell viability was maximum (5.8×10^7) in experimental tomato plants compared to control (5.3×10^7) as shown (**Table 1**). This indicates that the formulation containing humic acid showed better support for the viable cells over a period of two months as compared to the control which had no humic acid added to it. **Fig. 3** represents the cell viability among the control and the experimental sets, which clearly depicts slightly higher cell viability in the experimental sets than the control sets.

The lab test also revealed that there was 100% inhibition of *Fusarium oxysporum* growth by the *Pseudomonas fluorescens* present in the formulation (**Table 1**). The formulation containing humic acid showed better support for the viable cells over a period of two months as compared to the control which had no humic acid added to it.

Field test

Studies revealed that the pots containing the formulation had better leafing characteristics in both crops, i.e. 52 leaves in case of tomato and 26 in case of radish (**Table 2**) after 4th week. The height of the stem was also increased in case of tomato plants (7.2 cm) as shown (**Table 3**). Fruiting occurred by fourth week

and the observations in the sixth week revealed 30% more number of fruits in the test plants. The protective properties of the liquid bio-formulation were tested in the laboratory as

well as in the field studies. This formulation effectively inhibits the Wilt of tomato caused by *Fusarium oxysporum* sp lycopersici (Fig. 4 to Fig. 6 and Table 4).

Table 1 : Showing cell viability at an interval of 15 days

Days	Humic Acid	Control
0 Day	10 ⁸	10 ⁸
15 Days	6.5x 10 ⁷	6.0x10 ⁷
30 Days	6.1x10 ⁷	5.9x10 ⁷
45 Days	5.8x10 ⁷	5.3x10 ⁷
60 Days	5.2x10 ⁷	4.9x10 ⁷

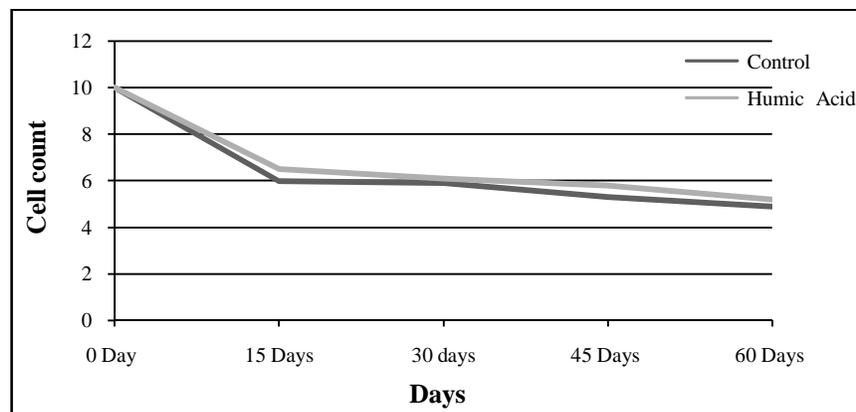


Fig. 3 : Graph comparing the cell count at a regular interval for the test and the control

Table 2 : Comparison of the number of leaves developed among the two plants after applying the bioformulation

Week	Tomato		Radish	
	Control	Test	Control	Test
1	00	00	00	00
2	06	09	10	15
3	18	21	12	20
4	41	52	15	26
5	50	100	25	40

Table 3 : The plant height recorded among the test and control varieties of tomato.

Crop	Parameter	Week	Control	Test
Tomato	Plant height (in cm)	1	0	0
		2	2	3.2
		3	3.5	4.8
		4	6	7.2
		5	7.9	9.1

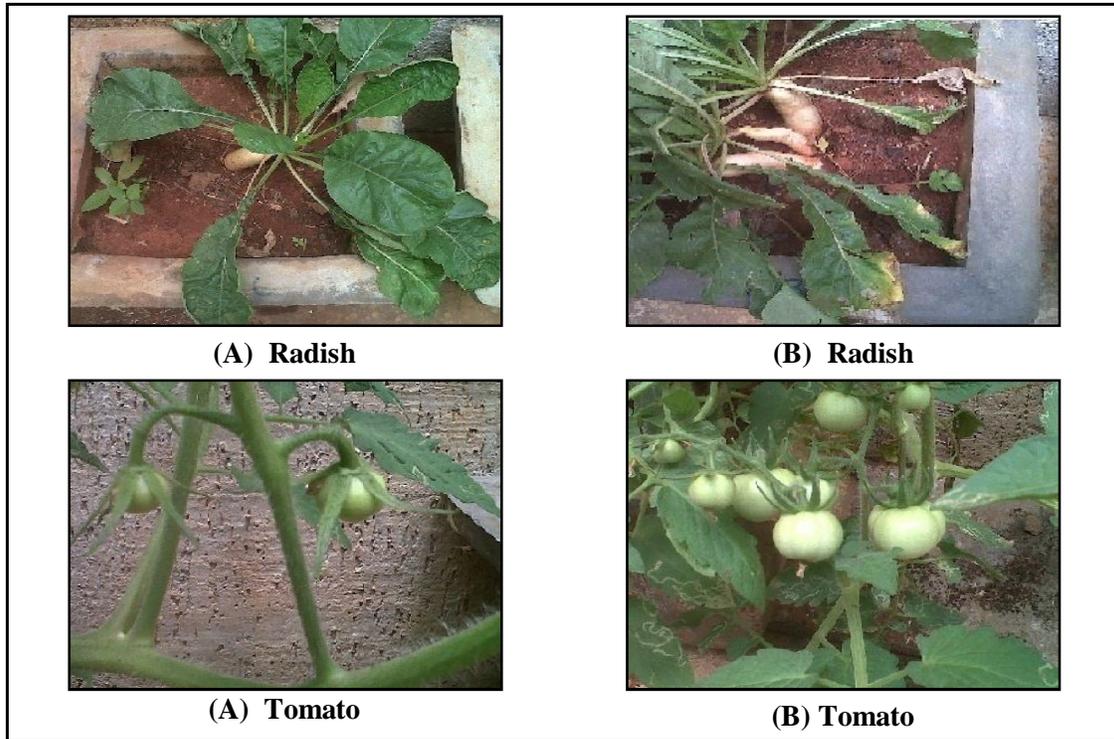


Fig. 4 : Shows the pot cultures for the A control and B test (containing prepared bioformulation) for radish and tomato plant

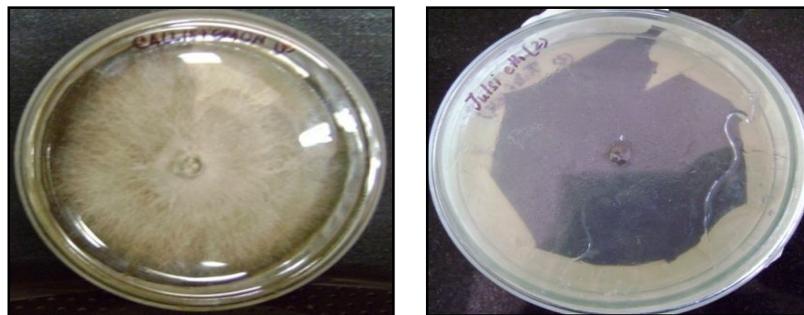


Fig. 5 : Shows plates comparing the radial growth of *Fusarium oxysporum* in the control and test plates respectively



Fig. 6 : Pot culture studies of tomato plant showing protective property of the prepared formulation against fungal pathogens

Table 4 : Percentage inhibition of *Fusarium*

Media	Average radii(cm)	% inhibition
Fusarium+ PDA medium	2.5	0
Fusarium+ PDA medium + 1ml bio-formulation (test)	No growth	100

CONCLUSION

The prepared liquid bio-formulation can be tested for crop varieties other than tomato and radish and also can be tested for its efficiency in inhibiting the growth of other fungal pathogens on various crop varieties.

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