

INNOVATIVE STRATEGY USING ENDOPHYTES FOR EFFECTIVE BIOCONTROL OF INSECT PESTS IN COTTON

ABSTRACT

Novel methods were developed using biochar and cowdung to enhance the efficacy of endophyte colonization in cotton plants. Aphids were used as test insects for this experiment. Aphids recovered from the endophyte treated plants were found to be affected by the muscardine disease. Cotton seedlings from seeds coated with *Beauveria bassiana* in a slurry of biochar plus cowdung and seedlings inoculated with endophyte *B.bassiana* using biochar plus cowdung were the most effective in causing disease in aphids.

INTRODUCTION

Insect pest problem in India

- There are about four major insect species which cause severe economic damage of 15-85% to cotton crop in India¹.
- Though cotton occupies only 7% of India's agricultural area, the crop is sprayed with 30-50% of the insecticides used in agriculture.
- The use of chemical insecticides leads to ecological disruption and severe environmental pollution.
- There is a need to strengthen ecofriendly biological control to move away from chemical intensive farming.

BACKGROUND RESEARCH

Endophytic insect pathogen as an alternative

- We found from literature² that an entomo-pathogenic non clavicipitaceous fungus called *B.bassiana* infects and causes the deadly muscardine disease specifically in a wide range of insect pests of cotton crop, but is safe to plants, animals and humans³.
- Recently, *B. bassiana* was also reported to occur inside the plant as an endophyte , though sparsely distributed³.
- We felt that cotton crop could be protected from a wide range of insects all through the season without the need of chemical pesticides if endophytic colonization efficiency is improved through indigenous innovation.

HYPOTHESIS

Cotton seeds and seedlings can absorb *B. bassiana* more effectively when delivered through friable biological material such as biochar (carbon rich) + cow-dung (nitrogenous manure). Biochar + cow dung provide an aerated matrix for seeds to absorb *B. bassiana*. Biochar + cow dung improvise the soil porosity, structure and texture of the root zone to significantly help the uptake of *B. bassiana* by seedlings.

GOAL OF STUDY

To enhance *B.bassiana* endophyte colonization in the cotton plant using naturally available biological material such biochar and cowdung

MATERIALS

- ***B.bassiana*** – Muscardine disease affected insects were collected in cotton fields of ICAR-CICR, and the fungus cultured on potato dextrose agar (PDA). *B.bassiana* was identified through microscopic examination.
- **Biochar** – Biochar was prepared from charcoal pellets made out of agricultural waste.
- **Cowdung** – Fresh cowdung was collected from cattleshed of ICAR-CICR
- **Autoclaved soil**

- Cotton seeds (var:Suraj) – acid delinted seeds
- Muslin cloth cages
- Cotton aphids, *Aphis gossypii* sourced from insectary of ICAR-CICR

METHOD OF EXPERIMENTATION

Preliminary experiments were conducted to find out 1. If *B. bassiana* could infect insects as an endophyte and 2. If biochar improved inoculation efficiency of *B. bassiana*.

Inoculating plants with *B. bassiana* using biochar

B. bassiana culture at 1×10^6 spores /ml was prepared and used in the experiments.

Assessment of different methods for endophytic colonization of *B. bassiana*

Sowing was done in pots with the following treatments:

Treatments	Pot-A	Pot-B	Pot-C	Pot-D	Pot-E
Biochar near root zone		√	√		√
<i>B. bassiana</i> near root zone			√	√	√
Seeds soaked in <i>B. bassiana</i>				√	√
Foliar spray of <i>B. bassiana</i> on 3 week old plants				√	√

Aphid bioassay:

1. Twenty five healthy second instar aphids were released per leaf on four leaves of two seedlings (5 wk old).
2. The potted plants were kept inside cages 60"x36"x36" lined with muslin cloth.
3. Observations were recorded 14 days after releasing aphids on the plants.
4. Leaves were examined for the endophytic presence of *B. bassiana* and infected larvae were confirmed for the presence of endophytic *B. bassiana*.

Statistical Analysis of Variance: Statistical analysis of aphid survival/recovery data was conducted through single factor analysis of variance (ANOVA)

Effect of inoculation methods of endophytic *B. bassiana* on survival of aphids on cotton plants

Pots	Treatments	Survival/recovery of aphids					Aphids recovered
		N*	R1	R2	R3	R4	%
A	Control seedlings	100	19	21	14	14	68
B	Biochar soil application	100	30	37	8	9	84
C	<i>B. bassiana</i> soil application with Biochar	100	14	16	8	8	46
D	<i>B. bassiana</i> : seed treatment + Foliar spray + soil application	100	2	2	5	5	14
E	<i>B. bassiana</i> : seed treatment + Foliar spray + soil application with biochar	100	0	0	0	0	0

*N= number of aphids released per treatment

Analysis of Variance (anova)

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	20012.8	4	5003.2	6.270722	0.00358	3.055568
Within Groups	11968	15	797.8667			
Total	31980.8	19				

The treatment differences are significant at 1% level of significance

RESULTS

1. Leaves on control plants did not contain *B. bassiana* and aphids were healthy.
2. Leaves of inoculated plants contained *B. bassiana* internally as endophyte and reduced aphid numbers by 38% less than control.
3. Application of *B. bassiana* with biochar near root zone significantly enhanced 'aphid-control' efficacy of seed treatment plus foliar application.
4. Results clearly indicated that the treatment effects were statistically significant at 1.0% level of significance.

PLAN OF EXPERIMENTS

Experiments were conducted in 20 pots each with the following treatments:

	Seed treatment				Root zone application for 3 wk old plants			
	Pot-A	Pot-B	Pot-C	Pot-D	Pot-A1	Pot-B1	Pot-C1	Pot-D1
Biochar + cowdung		√		√		√		√
<i>B. bassiana</i>			√	√			√	√

APHID BIOASSAY:

1. Fifty healthy second instar aphids were released per leaf on two leaves of each 5 wk old seedling.
2. The potted plants were kept inside cages 60"x36"x36" lined with muslin cloth.
3. Observations were recorded 14 days after releasing aphids on the plants.
4. Leaves were examined for the endophytic presence of *B. bassiana* and infected larvae were confirmed for the presence of endophytic *B. bassiana*.

RESULTS AWAITED:

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1. ICAR-CICR Web site data
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