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Studies on the impact of BT cotton on earthworm growth and reproduction (*Eisenia fetida*)

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Abstract

This study evaluated the effects of Bt cotton residues on *Eisenia fetida* reproduction, growth, and soil physicochemical factors. Results showed significant reductions in cocoon production, hatchability, growth, and survival in Bt treatments compared to non-Bt controls. Alterations in soil pH, EC, and nutrient status suggest that Bt residues, along with Cry protein exposure, impact earthworm health and soil properties.

Keywords: Bt cotton, *Eisenia fetida*, soil properties, reproduction, growth, Cry proteins

Introduction

Genetically engineered Bt cotton, designed to express *Bacillus thuringiensis* (Cry) proteins, is primarily used for its ability to control lepidopteran pests, reducing the reliance on chemical pesticides. However, the ecological implications of Bt crop residues in soil ecosystems have raised concerns, especially regarding non-target soil organisms. Earthworms, particularly *Eisenia fetida*, are well-established bioindicators of soil health due to their sensitivity to pollutants and crucial roles in nutrient cycling and soil structure. Previous studies have indicated that Cry proteins can persist in soil and potentially affect non-target organisms through ingestion or contact (Saxena and Stotzky, 2001; Zwahlen *et al.*, 2003) ^[1, 2].

This study aims to evaluate the effects of Bt cotton residues (leaves, stems, and roots) on the reproductive and growth parameters of *Eisenia fetida*, along with changes in key soil physicochemical properties. Understanding these interactions is critical to assess the long-term sustainability and ecological safety of genetically modified crops.

2. Materials and Methods

Earthworm Culturing and Maintenance

Eisenia fetida specimens were cultured and maintained under standardized OECD guidelines. The earthworms were kept in a controlled environment with optimal temperature (25 ± 2 °C) and moisture (60% of water holding capacity), using pre-composted cow dung as the substrate.

Soil Collection and Preparation

Sandy loam soil was collected from the Medak region of Telangana, India. Collected samples were sealed and brought to the laboratory. The chemical properties of soil samples were determined by following standard protocols. Soil pH and EC were analyzed by using pH meter and EC meter with 1:2 and 1:2.5 soil water suspensions respectively. Soil organic carbon was determined by using Walkley and Black method ^[3]. Available nitrogen was analyzed by alkaline permanganate method ^[4]. Available phosphorus was analyzed by using sodium bicarbonate method ^[5]. Available potassium was analyzed by using neutral normal ammonium acetate method ^[6].

Experimental Design

Bt and non-Bt cotton residues (leaves, stems, and roots) were dried, chopped, and mixed with the soil at a concentration of 5% w/w. Each treatment was replicated three times, including control (no cotton residues). The earthworms were introduced to the treated soil and monitored over a 60-day period.

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Reproductive parameters such as cocoon production, hatchability, juvenile biomass, and time to sexual maturity were recorded. Survival rates were assessed at the end of the exposure period. Soil parameters (pH, EC, organic carbon, available NPK) were analyzed before and after the experiment. Data was analyzed using SPSS software. Means were compared using t-tests and ANOVA ($p < 0.05$).

Results

Results of the present study was presented in Tables 1, 2 and figure-1 and 2, it showed that there were no significant differences in the weight and mortality of earthworms when treated with Bt and non-Bt cotton plants parts in

experimental field. The average weight, numbers of cocoons and Juveniles of *E. fetida* in non Bt cotton are slightly more than that of Bt cotton but the differences was not significant. The survival rate of earthworms in Bt cotton is 10% lesser rather than that of non Bt cotton.

The experimental findings showed varied impacts of Bt and non-Bt cotton residues on *E. fetida* reproductive and growth performance. While there were no statistically significant differences in weight and mortality between Bt and non-Bt treatments, a general trend of reduced cocoon production, hatchability, juvenile growth, and survival was observed in Bt-treated groups.

Table 1: Impact of Bt cotton residues on reproductive and growth parameters of *Eisenia fetida*

Treatment	Cocoon Production	Hatchability%	Juvenile Growth (mg)	Sexual Maturity (Days)	Survival Rate%
Non-Bt Leaves	22 ± 2	93.2 ± 1.4	345 ± 15	38 ± 2	98
Bt Leaves	15 ± 1.5	82.6 ± 1.8	298 ± 12	46 ± 3	88
Non-Bt Stems	21 ± 2	92.5 ± 1.6	340 ± 14	39 ± 2	97
Bt Stems	14 ± 1.3	80.4 ± 2.0	290 ± 10	47 ± 3	85
Non-Bt Roots	20 ± 1.7	91.8 ± 1.7	338 ± 16	40 ± 3	96
Bt Roots	13 ± 1.2	78.9 ± 2.5	285 ± 13	49 ± 4	84

Table 2: Soil Physicochemical Properties Pre- and Post-Exposure to Bt and Non-Bt Cotton residues

Treatment	pH	EC (μS/cm)	Org. Carbon (%)	Available N (kg/ha)	P (kg/ha)	K (kg/ha)
Initial Soil	7.2	205	0.65	112	18	245
Bt Residue (Post)	6.7	245	0.72	105	16	232
Non-Bt Residue (Post)	7.1	220	0.69	110	17.5	240

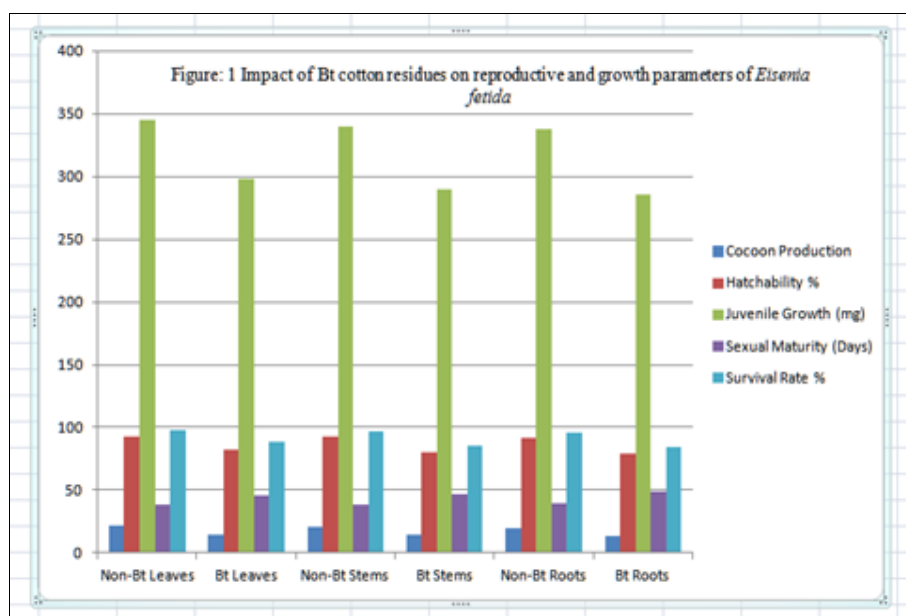


Fig 1: Impact of Bt cotton residues on reproductive and growth parameters of *Eisenia fetida*

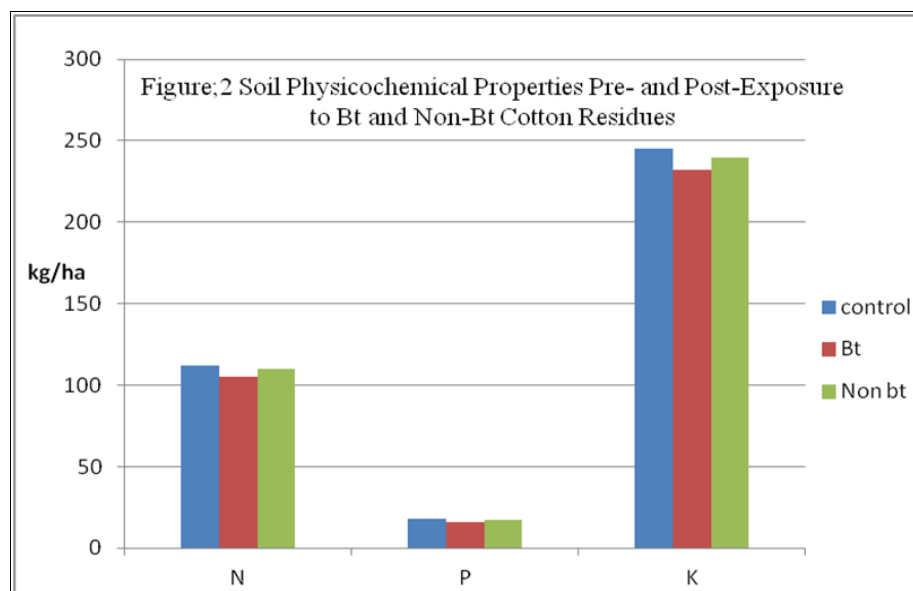


Fig 2: Soil physicochemical properties pre- and post-exposure to Bt and non-Bt cotton residues

A statistically significant reduction was noted due to Bt residues in cocoon production, hatchability, juvenile growth, and survival, and significantly delayed sexual maturity ($p < 0.001$). There was a decrease in soil pH from 7.2 to 6.7 under Bt residue addition, whereas a minimal reduction to 7.1 was observed in the Non-Bt residue treatment. Electrical conductivity (EC) changed substantially under Bt residues ($205 \rightarrow 245 \mu\text{S}/\text{cm}$, +19.5%), while the increase was minute in the Non-Bt treatment ($205 \rightarrow 220 \mu\text{S}/\text{cm}$, +7.3%). Organic carbon levels too increased ($0.65 \rightarrow 0.72\%$) while a dip in its level in Non-Bt residues ($0.65 \rightarrow 0.69\%$). The levels of N, P and K showed a reduction due to Bt impact, N levels values were ($112 \rightarrow 105 \text{ kg}/\text{ha}$, -6.3%), ($112 \rightarrow 110 \text{ kg}/\text{ha}$, -1.8%) under Bt and non Bt impact respectively. Available P decreased from 18 to 16 kg/ha under Bt residues (-11.1%), while Non-Bt residues caused only a slight decrease ($18 \rightarrow 17.5 \text{ kg}/\text{ha}$, -2.7%). K levels were ($245 \rightarrow 232 \text{ kg}/\text{ha}$, -5.3%) than under Bt and Non-Bt residues impact ($245 \rightarrow 240 \text{ kg}/\text{ha}$, -2.0%).

Discussion

Based on our present findings, the study revealed that Bt cotton toxin affects the earthworms at different developmental stages, it may be related to cry protein toxin or the nutrients present in the soil. Earthworms have been adapted to respond to stress conditions. Survival, growth and reproduction are also influenced by physical and chemical factors of the Soil such as pH, organic matter content and mineral availability. The successful survival of *Eisenia fetida* is due to its potential detoxification capability. But several studies reveal that Bt protein had no toxicological risk on non target organisms. Our findings confirm that Bt cotton residues adversely affect the reproductive success and growth of *Eisenia fetida*, consistent with earlier studies that reported similar negative impacts from Bt crop residues (Zwahlen *et al.*, 2003; Jansch *et al.*, 2005; Schrader *et al.*, 2008) [7, 8, 9]. Changes in soil physicochemical factors, particularly lowered pH and altered nutrient dynamics, may exacerbate biological stress [10, 11, 12, 13, 14] ((Zhang *et al.*, 2016). Reduced earthworm fitness implies risks to soil structure and nutrient cycling in Bt cotton systems (Hilbeck *et al.*, 1998). Similar

observations were made by Shen *et al.*, (2006) [11], Singh *et al.* (2012) [12], and Griffiths *et al.*, (2005) [13].

Conclusion

Bt cotton residues can negatively affect both earthworm reproduction and growth, as well as alter soil chemical balance. These results suggest the need for ecological safety monitoring of genetically modified crop residues, especially in soil systems reliant on earthworm-mediated processes. Furthermore, it was concluded that many factors shows impact on earthworm growth and reproduction when exposed to Bt cotton in comparison to non Bt cotton. Further we recommend to carry out the research work to know the factors which show huge impact on earthworm growth and reproduction in Bt cotton rather than non Bt cotton.

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