TO STUDY THE EFFECT OF BT (*Bacillus thruingiensis*) COTTON CULTIVATION ON SOIL HEALTH

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BY

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CERTIFICATE

I declare that the dissertation entitled “TO STUDY THE EFFECT OF BT *(Bacillus thuringiensis)* COTTON CULTIVATION ON SOIL HEALTH” has been prepared by me under the guidance of Dr. Sunil Mittal, Assistant Professor, Centre of Environmental Science and Technology, School of Environment and Earth Sciences, Central University of Punjab. No part of this dissertation has formed the basis for the award of any degree or fellowship previously.

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ABSTRACT

To Study the Effect of Bt *(Bacillus thuringiensis)* Cotton Cultivation on Soil Health

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Key Words: Transgenic; Soil Enzymes; Cotton; Soil Sample; Significant; *Bacillus thruingiensis;* Biochemical properties; Agricultural Field; Soil Sample.

The impact of transgenic *Bacillus thruingiensis* (Bt) cotton cultivation on soil enzymes and physico-chemical parameters of soil were investigated. Soil samples were collected from surface (0-20 cm depth) of agricultural fields near Bathinda, District. Where Bt cotton (RHC134) and non Bt cotton variety had been continuously cultivated for last two years. Soil samples were collected after harvesting of cotton crop. A control sample was collected from the adjoining waste land where no crop was grown from last many years. To observe effect of Bt cotton on soil biochemical properties, activities of soil enzymes such as amylase, cellulase, urease, dehydrogenase, alkaline phosphatase and acid phosphatase were assayed. Statistically significant enhancement in activities of the above enzyme was observed in Bt cotton soil samples as compared to non Bt cotton soil samples. No difference was observed in cellulase activity between Bt and non Bt cotton soil samples. Further, to study the effect of Bt cotton on physico-chemical properties, pH, conductivity, texture, total organic carbon (%) and organic matter (%), available nitrogen and available phosphorous content were estimated. In conclusion, significant changes were observed in Bt cotton grown soil samples.

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(Manohari Kumari)

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF ABBREVIATIONS

Sr.No.	Full form	Abbreviation
1.	Genetically modified	GM
2.	*Bacillus thuringiensis*	Bt
3.	Crystal	Cry
4.	Hectares	ha
5.	Gram	g
6.	Milliliter	ml
7.	Microns	µ
8.	Nitrogen	N
9.	Phosphorous	P
10.	Molar	M
11.	Nanometer	nm
12.	Hour (s)	hr (s)
13.	Potassium dichromate	$K_2Cr_2O_7$
14.	Ferrous sulphate	$FeSO_4$
15.	Potassium permanganate	$KMnO_4$
16.	Ultra violet-visible	UV-VIS
17.	Minutes	m

Chapter 1

Introduction

Genetic engineering is a powerful technology used to incorporate desired traits in an organism. The technology is in highlights for its promising use in sustainable development of agricultural system. New transgenic crop plants with high yield and improved resistance against pests, pathogens, parasites etc. are the outcome of this technology (Pickrell, 2006). The genetic modification of crop plants has beneficial effects in economic, agronomic and environmental fields. However, the long-term impacts of genetic modifications on human health and environment are still controversial. The serious issues like the development of super pest, gene pollution, effects on non- target soil microbes and physico-chemical properties require more research.

Genetic modification involves the introduction of foreign DNA or synthetic genes into the organism of interest for the desired character. Genetically modified crops are classified into three generations:

1. First generation of genetically modified crops, provide protection against insects and/or resistance to herbicides. There are also fungal and virus resistant crops developed or in development.
2. Second generation of genetically modified crops developed to aim directly improve yield. These crops are salt, cold or drought tolerant and with increased nutritional value (Deborah and Whitman, 2000).
3. Third generation consists of pharmaceutical crops that contain edible vaccines and other drugs (Marvier, 2008).

The major genetically modified crops grown throughout the world are soybeans, corn, cotton, alfalfa, Hawaiian papaya, tomatoes, canola, sugarcane, sugar beet, rice, squash and sweet peppers.

Due to usefulness of Bt. Cotton, it has been introduced as crop of choice in place of conventional cotton in many parts of the world. According to the International Service

for the Acquisition of Agri-biotech Applications (ISAAA), worldwide, GM cotton was cultivated on an area of 16 million hectares in 2009. This was 49% of the total worldwide area planted under cotton. In 2009, the US cotton crop was 93% genetically modified, the Chinese cotton crop was 68% genetically modified and 95% of the Australian cotton crop was genetically modified. The five leading exporters of cotton in 2010 are (1) The United States, (2) India, (3) Brazil, (4) Australia, and (5) Uzbekistan (Source: USDA-Foreign Agriculture Service).

Bacillus *thuringiensis* (Bt) cotton is the only genetically modified crop which was introduced in India in 2002. Following its success, the area under Bt cotton in India continues to grow at a rapid rate, increasing from 50,000 hectares in 2002 to 8.4 million hectares in 2009. The total cotton area in India was 9.6 million hectares (the largest in the world or, about 35% of world cotton area), so GM cotton was cultivated on 87% of the cotton area in 2009. This makes India as the country with the largest area of GM cotton in the world, surpassing China (3.7 million hectares in 2009). In India, the states of Maharashtra (26.63%), Gujarat (17.96%), Andhra Pradesh (13.75%) and Madhya Pradesh are the leading cotton producing states. Cotton is a major cash crop of southwestern regions of Punjab i.e. Bathinda, Faridkot, Firozepore, Mansa, Moga, Mukatsar and Sangrur. Bathinda has 141 thousand ha area under cotton cultivation followed by Ferozepur 140 thousand ha area. In Punjab, cotton is grown under irrigated condition. Punjab contributes 8 to 13 per cent of the national cotton production. Earlier, area in Punjab under cotton cultivation was covered by conventional cotton. But after the introduction of Bt cotton in 2005-06 the entire conventional cotton area has been shifted to Bt hybrids and during 2007-08 more than 86% cotton area was under Bt Cotton cultivation. (Revolution in Indian Cotton.Govt of India, 2009)

Cotton is a flowering plant, in the family Malvaceae and belongs to genus *Gossypium*. The plant is a biennial shrub and native to tropical and subtropical regions around the world, including the Americas, Africa, and India. In addition, is most widely used natural fibre in clothing (Metcalf, 1999). The botanical purpose of cotton fibre is to facilitate seed dispersal. For successful cultivation of cotton, the favourable

environmental conditions are long frost-free period, plenty of sunshine and a moderate rainfall. In general, seasonally dry tropics and subtropics in the Northern and Southern hemispheres are favourable for cotton growth; however, with increased facilities of irrigation, it is even cultivated in less rain fed parts of the world.

The main problems associated with conventional cotton were fewer yields due to higher intensity of insect and pest attacks and it required high prices of agricultural inputs (pesticides, fertilizers etc.). To overcome these problems Bt cotton had been introduced.

Bt cotton had been developed through the transfer of a gene from a soil bacterium, *Bacillus thuringiensis*. This gene, when expressed in cotton plant, produces insecticidal Bt protein, which is harmful to the larvae of moths, butterflies, beetles, and flies. When insects feed on the plant, the toxin enters the body and the alkaline pH of their digestive tract activates the toxin. Bt toxin is inserted into the insect gut cell membrane, forming a pore. The pore results in cell lysis and eventual death of the insect (Babu and Geetha, 2008).

The popularity of transgenic varieties (such as Bt cotton) among the farmers is due to two reasons: the potential of increase in yield and savings on pesticides and labor resulting from fewer pest attacks. Bt cotton is genetically enhanced to resist three bollworms: cotton bollworm (*Helicoverpa armigera*), the spotted bollworm (*Earias insulana*), and the pink bollworm (*Pectinophora gossypiella*).

The area under cultivation of genetically modified crops is increasing day by day. As a result, the bio safety of these crops has been a major concern in recent years, and many studies related to effects of Bt cotton and other GM crops on soil health have been conducted (Men *et al*.,2003; Bai *et al*. 2003; Li *et al*., 2002; Liu *et al*., 2002; Zhang *et al*., 2000). Some studies revealed that transgenic Bt cotton has no harmful effects on soil health may even have beneficial effects, while other studies have reported some adverse effects (Cui and Xia 2000; Tan *et al*., 2002). Soil health refers to the capacity of a specific kind of soil to function as a vital living system to sustain plant and animal productivity. The basic assessment of soil health and soil quality is

necessary to evaluate the degradation status and changing trends following different land use and smallholder management interventions (Lal and Stewart, 1995).

Soil health Indicators, however, will vary according to the location, and the level of sophistication. There are three main categories of soil indicators.

1. Biological indicators of soil quality that are commonly measured include soil organic matter, respiration, microbial biomass (total bacteria and fungi), soil enzymes and mineralizable nitrogen.
2. Chemical indicators of soil health measured are pH, temperature, soil nitrate, alkalinity, acidity and electrical conductivity of the soil.
3. Soil physical properties are estimated from the soil's texture, bulk density (a measure of compaction), porosity, water-holding capacity (Hillel, 1982). Balance between chemical, physical and biological components contribute in maintaining soil health.

Evaluation of soil health therefore requires study of all these components. Soil enzymes are most eligible candidate among the biological indicators.

Soil enzymes are a group of enzymes, inhabiting soil. In the soil, enzymes are released from micro-organisms, plants, invertebrates and other animals, which constitute the soil biota. Soil biota mediates or regulates a variety of functions essential for plant growth and productivity, soil resource structure, and ecosystem health. Any change in crop residue and rhizosphere inputs will potentially modify the dynamics of soil biota composition and activity (Gupta *et al*., 1998 and1999). Enzymes are required to maintain the soil health by key biochemical functions like organic matter decomposition (Sinsabaugh *et al*., 1991), catalysing several vital reactions necessary for the life processes of microorganisms in soils, decomposition of organic wastes, nutrient cycling, and hence playing an important role in agriculture (Dick *et al*., 1994 and 1997). All soils have different levels of different enzymes depending upon factors like organic matter content of the soil, composition and activity of biotic community of the soil. The main soil enzymes are amylase, arylsulphatases, β-glycosidase, cellulase, dehydrogenase, phosphatase, protease, and urease released from plants (Miwa *et al*., 1937), animals (Kanfer *et al*., 1974), organic compounds, and microorganisms (James *et al*., 1991; Richmond, 1991;

Shawale and Sadana, 1981) and soils (Gupta *et al*., 1993; Ganeshamurthy *et al*., 1995).

Soil enzyme activities are used as the indicators for measuring the degree of soil degradation due to soil pollution and different practices. Soil enzyme activities (1) are closely related to soil organic matter, soil physical properties and microbial activity or biomass, (2) changes, much sooner than other parameters, thus providing early indications of changes in soil health (Dick *et al.,* 1996).

Climatically, India is a subtropical country. Thus as compared to temperate and sub temperate countries, biological and biochemical response of Indian soil to increasing cultivation of Bt cotton may vary. Therefore, we have studied the effects of Bt cotton cultivation on soil enzyme activities and physical-chemical parameters of soil from agricultural fields near Bhatinda, District. An evaluation of the ecological risks of Bt cotton was made on the basis of changes in enzymes activity and physical-chemical parameters of the soil. However little experimental data is available on the environmental consequences of toxins released during and after plant development. No much work has done yet in India on effects of genetically modified crops on soil health.

Chapter 2

Review of literature

2.1. Effect of different crop practices on soil health

The diversity and activity of soil biota is affected by various agricultural practices like exotic plant species, fertilizers, water stress, field management, nitrification, grassland improvement and soil depth etc. Ajwa *et al*. (1999) studied that long-term burning practice significantly (P<0.05) increased activities of urease and acid phosphatase but decreased the activities of β-glucosidase, deaminase and alkaline phosphatase. Also long-term nitrogen fertilization significantly cause increased activities of β-glucosidase and acid phosphatase but decreased urease activity. In Asia, adverse effects on soil health and soil quality arise from nutrient imbalance in soil, excessive fertilization, soil pollution and soil loss processes (Zhang *et al*., 1996; Hedlund *et al*., 2003)

Gianfreda *et al*. (2004) studied about effect of intensive agricultural practices and organic pollution on soil enzyme activities and physical-chemical properties of the soil. According to them, as compared to agricultural soils, non-cultivated soils heavily or moderately polluted by organic contaminants showed much lower values or complete absence of enzymatic activities. Koenning and Barker (2004) found that cotton fields containing high nematode diversity, which is influenced by different agricultural practices such as tillage, use of pesticides and fertilizers.

Hannula *et al*. (2010) concluded that any change in plant genotype like the GM trait under study, showed no lasting effect on soil fungal communities. Although due to this modification, there might have been changes in root exudates composition expected and by measuring this; we can evaluate the possible effects of genetically modified crops on soil fungal community and other soil community structure. Moreover, insight in the community structure of soil fungi is not always sufficient to determine the functionality of the fungal community (Hanson *et al*., 2008).

2.2. Release of Bt toxin in soil

Insect-resistant genetically modified crops (Bt crops) release Bt-toxin i.e. Cry proteins during their growth and after it on the soil surface and in rhizospheric soil from root

exudates. These proteins are not stable in soil and in different environmental conditions. However, due to repeated cultivation of GM crops. The concentration of Bt toxin increased in the soil. (Tabashnik, 1994; Crecchio and Stotzky, 1998; Tapp and Stotzky, 1998; Saxena and Stotzky, 2001; Saxena and Stotzky, 2001; Saxena *et al*., 2002; Zwahlen *et al*., 2003; Muchaonyerwa *et al*.,2004; Stotzky, 2000; Stotzky, 2002 and Stotzky,2004). This lead to affect the composition and activity of soil microbial communities (Tapp and Stotzky, 1995; Crecchio and Stotzky, 1998; Tapp and Stotzky, 1998; Stotzky, 2000; Crecchio and Stotzky, 2001). Bt maize, Bt rice and potato release Cry proteins to soil in root exudates throughout the growth of the plant (Saxena *et al*., 1999; Saxena *et al*., 2004; Saxena and Stotzky, 2000; Icoz and Stotzky, 2007). Bt-toxin from Bt cotton plants released into the soil through two pathways, i.e., biomass incorporation and root exudates (Saxena and Stotzky, 2001; Mina *et al.,* 2008; Liu, 2009).

Watson and Gupta (2004) studied that Bt toxin enter the soil system throughout the cotton growing season. Thu (2004); Baumgarte and Tebbe (2005) confirmed the release of Cry protein in root exudates continued throughout growth, and levels of the protein in soil did not correlate with a specific period of plant growth. The continuous release, via root exudates, leads to higher concentrations of Cry protein in rhizosphere than in bulk soil. The rhizosphere community may be significantly altered by changes in root exudates of transgenic plant (Brusetti *et al*., 2004). In most of the cells of a Bt transformed plant Bt proteins are present as active toxins and so are present in all plant parts and residues, and may be released into the soil through various routes depending on the crop and environment (Mendonca *et al*., 2006).

Sun *et al.* (2006) conduct their study on leaves and stems of transgenic Bt cottons; Guo-Kang 12 (Bt-GK) and Zhong-Kang 30 (Bt-ZK). Non Bt cotton Zhong- Mian 30 (non Bt-ZM) was used as the control. The soil treated with Bt cotton tissues had a significantly higher Bt toxin content and there was rapid decrease in toxin content in the first 7 days of incubation, but after it there was decrease in the toxin degradation rate and the Bt content remained almost unchanged after 28 days. The Bt toxin content was still high (14.19–22.69 ng g−1 soil) by the end of incubation i.e. after the

56th day, representing 40.79% (Bt-ZK) and 59.98% (Bt-GK) of its initial introduced amounts.

Dong *et al*. (2006) concluded that the efficacy of transgenic Bt cotton against target pests varies according to plant age, plant part or structure. Bt toxin expression is also affected by environmental stresses such as high temperature, heavy drought, water logging, elevated CO_2, nitrogen deficiency, rational nitrogen fertilization and timely irrigation.

Helassa *et al*. (2011) studied about the fate of Cry 1Aa Bt toxin in contrasting soils after different treatments. The toxin was efficiently extracted from each soil sample using an alkaline buffer containing a protein, bovine serum albumin, and a nonionic surfactant, Tween 20. There was marked decline in extractable toxin after incubation of weeks to months. In addition, it was soil-dependent. The decrease of extractable toxin with incubation time was not related to microbial degradation but mainly to physicochemical interactions with the surfaces that may decrease immunochemical detectability or enhance protein fixation. Hydrophobic interactions may play an important role in determining the interaction of the toxin with surfaces.

2.3. Effect of Bt toxin on soil health

The area under cultivation of genetically modified crops is increasing day by day. As a result, the bio safety of these crops has been a major concern in recent years, and many studies related to effects of Bt cotton and other GM crops have been conducted (Men *et al*.,2003; Bai *et al*. 2003; Li *et al*., 2002; Liu *et al*., 2002; Zhang *et al*., 2000). Most studies revealed that transgenic Bt cotton has no harmful effects on soil animals or plants and may even have beneficial effects, while other studies have reported some adverse effects (Cui and Xia 2000; Tan *et al*., 2002). Microbial processes have been shown to be particularly responsive to protein substrates (Wheatley *et al*., 2001).

Turrini *et al*. (2004) and Castaldini *et al*. (2005) observed that fungi appear to be most affected organisms by Cry proteins in soil. In their experiments, the roots of *Bt* maize (event 176) were less colonized with mycorrhizae than their non-*Bt* near-isogenic counterpart. Therefore, *Bt* maize may not only lose an important symbiont that

contributes to plant nutrition, but the plants might be even more susceptible to insect pests because without mycorrhizae, maize attracts fewer natural enemies of the pests. The microbiota (e.g., bacteria, actinomycetes, fungi) associated with residues of Bt cotton were significantly different from those associated with residues of herbicide tolerant cotton (Roundup Readys).

Griffiths *et al*. (2005) observed significantly lower natural population of nematodes in the field with Bt maize than with non Bt maize. Rui *et al*. (2005) found higher numbers of functional bacteria in the rhizospheric soil samples from non-Bt cotton (Shiyuan 321) than the soil samples from Bt cotton counterpart (NuCOTN99) after adding pure Bt toxin to soil.

Liu *et al*. (2007) compared seasonal effects of transgenic rice expressing the Cry1Ab insecticidal protein active against lepidoperan pests and the insecticide triazophos [3-(*o*,*o*-diethyl)-1-phenyl thiophosphoryl-1,2,4-triazol] on soil enzyme activities and microbial communities under field conditions. During a 2-year field study, rhizospheric soil samples from transgenic-Bt rice (Bt), non-Bt parental rice (Ck) and non-Bt parental rice with triazophos (Ckp) applied were taken at four stages i.e. seedling, booting, heading and maturing. On the application of triazophos there were found some occasional and inconsistent effects on the bacterial composition in the rhizospheric soil of rice plant at the booting and heading stages as compared with that of transgenic-Bt rice. The differences occurred were not statistically significant (P>0.05) in dehydrogenase activity, phosphatase activity, respiration, methanogenesis or fungal community composition in rhizospheric soil samples between Bt, Ck and Ckp over the rice cropping cycle under study. However, variations detected in the selected enzyme activities and microbial community composition in the rhizospheric soil of Bt, Ck and Ckp were seasonal. And hence the application of triazophos and KMD1 (Bt) rice expressing the cry1Ab gene showed no measurable adverse effect on the microbial community composition or on the key microbial processes in rhizopheric soil over 2 years of rice cropping.

Devare *et al*. (2007) conducted the study to determine the effect of Cry3Bb Bt maize with those of the insecticide tefluthrin on soil microbial biomass and activity in the field

over a 3-year cropping cycle. They concluded that neither the Bt maize nor the insecticide had adversely effects the microbial biomass, N mineralization potential, or nitrification and respiration rates. Vaufleury *et al*. (2007) studied the effect of Bt-maize (MEB307 expressing the insecticidal Cry1Ab protein) and a near isogenic non-Bt variety (Monumental) on the garden snail (*Helix aspera*), soil microarthropods (Collembola, Actinedida, Acaridida, Gamasida and Oribatida) and mycorrhizal fungi in a four month microcosm. They concluded that Bt protein expressed in Bt-maize is not toxic either directly or indirectly to the three non target group of soil organisms studied.

Hu *et al*. (2008) carried their study about the impact of multiple-year (0–5 years) cultivation of transgenic Bacillus thuringiensis (Bt) cotton on the functional bacterial populations in rhizospheric soil samples. Rhizospheric soil samples were collected at different stages of cotton like the seedling, squaring, flower and boll, and boll opening. Different cultivation-dependent approaches were applied for the measurement of numbers of bacteria involved in nitrogen fixing, organic phosphate dissolving, inorganic phosphate dissolving and potassium dissolving. They concluded that differences in the number of functional bacteria population between rhizospheric soil of Bt and non-Bt cotton in the same field or among the four fields were either transient or absent. The major conclusions from this study are: (1) There is no overall effect of repeated cultivation of transgenic Bt cotton expressing Cry protein on the number of functional bacteria; and (2) within one growing season, there had no clear effect on the number of functional bacteria in the rhizosphere soil. These results suggest that cultivation of Bt crops over multiple years probably poses little ecological or environmental risk. However, the results presented here should be considered preliminary because they used a culture- based technique (which detects only a small portion of the microbial community) and because they evaluated only a few functional types of bacteria.

Wenke Liu (2009) demonstrated that Bt crops (Bt cotton & Bt Rice) effect the soil ecosystem, micro-organisms as well as they change the soil biochemical properties.

Liu *et al*. (2010) studied the effects of genetically modified maize (*Zea mays* L.) expressing the *Bacillus thuringiensis* Berliner Cry1Fa2 protein (Bt) and phosphinothricin or glyphosate herbicide tolerance on soil chemistry (organic matter, N, P, K and pH), compared with non-GM controls. They observed high content of organic matter and total nitrogen in GM maize soil samples as compared to non GM soil samples. But this was opposite in case of total phosphorous i.e. phosphorous content was higher in non GM maize soil samples as compared to GM maize soil samples. Their results indicate that growing genetically modified crops instead of conventional crops may alter soil chemistry, but not greatly, and that effects will vary with both the soil type and specific genetic modification.

Njinju *et al*. (2011) carried out their field and laboratory studies about the effects of Bt cotton protein endotoxins on belowground fauna. The data generated in their study showed that the Bt toxin released by Bt cotton into the soil had no effect on *Steinernema karii* which is an important entomopathogenic nematode and is the part of belowground fauna biodiversity. However, they suggested that long-term experimental studies are necessary to get more data on belowground fauna. Belowground macro fauna other than the entomopathogenic nematodes should also be included in future studies such as the effect of Bt toxin on soil bacteria and fungi as they play major roles as belowground macro fauna.

Mina *et al*. (2011) reported that dehydrogenase, alkaline phosphatase, nitrate reductase and urease enzymes activity was high in Bt cotton rhizosphere as compared to non Bt rhizosphere. Differences in activity of alkaline nitrate reductase, urease and phosphatase enzymes between Bt and non Bt plants rhizospheric soil samples were not found statistically significant Except dehydrogenase enzyme. Significant differences ($P<0.05$) were observed in the number of nematodes, collembola and ants between the Bt and non Bt cotton rhizospheric soil samples. Number of nematodes, collembola and ants were more in Bt plants rhizospheric soil as compared to non Bt plants rhizosperic soil. While at flowering stage the Number of nematodes, collembola and ants were highest in Bt and non Bt cotton rhizospheric soil samples. At last they concluded that there was no adverse effect of Mech 162

variety of Bt cotton on soil biochemical and microbial indicators. The differences occurred by growing Bt cotton were not as large as those resulting from seasonal changes. Hence, the effect of Bt cotton on soil ecology was within normal variation expected in conventional agriculture.

2.4. Soil enzymes as an indicator

Soil enzyme activities are also proposed to be the indicators for measuring the degree of soil degradation as a result of soil pollution and different practices. Soil enzymes like urease, acid phosphatase, arylsulfatase, invertase, and cellulase activities play an important role in soil microbial activity, because they are related to some important N, P, S, and C reactions, respectively (Nannipieri *et al*. 1990; Deng and Tabatabai 1997; Kandeler *et al*. 1999; Nannipieri *et al*. 2002). The roles and activities of amylase may be influenced by different factors like cultural practices, type of vegetation, environment, and soil types (Ross, 1968; Ross and Roberts, 1970; Pancholy and Rice, 1973;Ross, 1975).

Soil dehydrogenase activity is also used to measure the degree of disruption caused by pesticides, trace elements and management practices. According to Burns (1978) the dehydrogenase enzyme activity is commonly used as an indicator of biological activity in soils. Burns (1982) studied the important role of soil proteases in the ecology of microorganisms in the ecosystem. Dick and Tabatabi (1992) studied that soil enzyme activities are often used as indices of microbial activity.

Frank and Malkones (1993) reported that dehdrogenase enzyme is used as a measure of any disruption caused by pesticides, trace elements or management practices to the soil as well as a direct measure of soil microbial activity. Studies have shown that activities of cellulases in agricultural soils are affected by several factors. These include temperature, soil pH, water and oxygen contents, chemical structure of organic matter (Deng and Tabatabai 1994; Alf and Nannipieri 1995), soil mineral elements (Deng and Tabatabai 1994) and the trace elements from fungicides (Deng and Tabatabai 1994; Arinze and Yubedee 2000). Arinze and Yubedee (2000) reported that fungicides benlate, calixin, and captan inhibited cellulase activity in *Fusarium monoliforme* isolates. Jepson *et al*. (1994) studied that soil enzyme activity like

urease, dehydrogenase, and phosphatases used as indicators to evaluate the impact of toxins on soil microbiological activity.

Acosta-Martı´nez and Tabatabai (2000); Madejo´n *et al*. (2001) concluded that β-Glucosidase enzyme is very sensitive to changes in pH, and soil management practices. Chapin *et al*. (2002) said that soil protease activity tends to mirror microbial activity. Kourtev *et al.* (2002) studied about the critical role of soil enzymes in catalyzing reactions necessary for organic matter decomposition and nutrient cycling. Phosphatases are synthesized and activated during phosphorous stress and plant growth. For example, when there is a signal indicating Phosphorous deficiency in the soil, acid phosphatase secretion from plant roots is increased to enhance the solubilization and remobilization of phosphate, thus influencing the ability of the plant to cope with Phosphorous stressed conditions (Karthikeyan *et al*., 2002; Mudge *et al*., 2002; Versaw and Harrison, 2002). More long lived and their activity in soil is correlated more strongly with availability of organic phosphate than microbial activity (Kroehler and Linkins, 1991). They are good indicators of soil fertility.

Wu *et al*. (2004) found that the phosphatase activity of paddy soils was not affected by the incorporation of Bt transgenic rice straw into the soil, whereas, the dehydrogenase activity was increased.

Biao Liu *et al.* (2005) reported that transgenic plants have been found to have significant effect on soil population of non-target bacteria and fungi (Donegan *et al*. 1995, 1999; Ahrenholtz *et al*. 2000), soil enzyme activities (Dongan *et al*. 1999, Giovani *et al*. 1999) and the structure of microbial community (Cowgill *et al*. 2002; Dunfield and Germida, 2001, 2003). Different studies on genetically modified crops by Wu *et al*. (2004);Flores *et al*. (2005); Shen *et al*. (2006); show different effects on activity of some enzymes like ureases, alkaline phosphatases, dehydrogenases, phenol oxidases and proteases in the soil of Bt and non Bt cotton. Flores *et al.* (2005) reported a slower degradation of *Bt* plants like canola, cotton, maize, potato, rice, and tobacco in the soil because of a higher lignin content in *Bt* plants, whereas there is no differences in decomposition or Nitrogen-mineralization between *Bt* and non-*Bt* crops according to other studies. Sometimes there are differences in the chemical

composition (e.g., the content of starch, soluble N, proteins, carbohydrates and lignin) of *Bt* maize hybrids from their isogenic non-*Bt* counterparts. For example, Masoero *et al*. (1999); Saxena and Stotzky (2001); Flores *et al*. (2005); Poerschmann *et al*. (2005) have been reported higher lignin content. Masoero *et al*. (1999); Saxena and Stotzky (2001) reported about lower lignin content (Escher *et al*., 2000), and according to Folmer *et al.* (2002); Jung and Sheaffer (2004); Mungai *et al*.(2005); Lang *et al*.(2006) no differences in lignin content have been reported.

Sun *et al.* (2006) concluded that the transgenic Bt cotton tissues and their degradation product had different effects on the tested enzyme activities of soil with a positive activities of soil urease, acid phosphomonoesterase, invertase and cellulase activity and a negative effect on arylsulatase activity. Yang *et al*. (2006) studied that urease activity in the soil is affected by many factors like organic matter content of the soil, soil depth, cropping pattern, heavy metals and environmental factors like temperature. Lupway *et al*. (2006) concluded that activity of dehydrogenase enzyme decreases with increasing frequency of Glyphosate resistance crops in both the rhizosphere and bulk soil.

Chapter 3

3.1 Objectives

1. To study physical-chemical parameters such as pH, conductivity, available phosphorous, available nitrogen, total organic carbon and organic matter of Bt cotton, non Bt cotton grown soil and control soil.

2. To study enzyme activity of different soil enzymes like amylase (EC 3.2.1.X), cellulose (EC 3.2.1.4), urease (EC 3.5.1.5), dehydrogenase (EC 1.1.1.X), alkaline phosphatise (EC 3.1.3.1) and acid phosphatise (EC 3.1.3.X), spectrophotometrically.

Chapter 4

Materials and Methodology

4.1. Instruments used

Instruments used during the course of this work were uv-vis spectrophotometer (Systronics, 2202), cooling centrifuge (Remi,CPR-24), water analyzer kit (Systronics, 371), Kjeldahl distillation unit (Khera, RI-145), incubator (NSW,IUS-4-15/K.WATT), test tube rotator (Tarsons, RR087), incubator shaker (Khera), weighing balance (Citizen, CX120), boiling water bath (JSGW, double walled 12 holes) and magnetic stirrer (Khera, KI-140).

4.2. Chemicals and reagents

Chemicals used were from SD fine- chem. Ltd., SRL, High media and Sigma Aldrich. The main chemicals used were 3, 5-Dinitrosalicylic acid solution, Carboxymethyl cellulose, 2,3,5-Triphenyl Tetrazolium Chloride, Triphenyl Formazan, p-nitrophenyl phosphate and p-nitro phenol.

4.3. Experimental site

Soil samples were collected from agricultural fields near Bathinda District of Punjab, where *Bacillus thruingiensie* (Bt) cotton variety (RCH134) and it's isogenic non Bt cotton variety had been continuously cultivated for two years. The soil samples were collected after the crop harvesting i.e. during November to February. Lab experiments were conducted at Central University of Punjab, Bathinda.

4.4. Soil sampling

Samples were collected from the top layer (0-20 cm) of soil in plastic bags. The collected soil samples were ground and sieved through 2 mm sieve and stored at -20ºC before analysis. After it, the soil samples for enzymatic assay were kept at 4ºC and for physical-chemical analysis kept at room temperature.

4.5. Enzymatic assay

Determination of soil amylase activity

Chemicals and Reagents

1. Toluene
2. Sorensen's buffer (pH 5.5, 0.06M)
3. 1% Starch solution
4. 3, 5-Dinitrosalicylic acid solution

Procedure

About 0.2 ml of toluene was added to 3 g of preserved (at 4ºC), screened soil and kept at room temperature for 15 minutes. After 15 min, 6 ml of Sorenson's buffer (pH 5.5, 0.06M) and 6 ml substrate solution (1% starch) were added to the flask, and the mixture was incubated at 30º C for 24 hours. After 24 hours content was centrifuged at 17, 000 rpm for 10 minutes. To 1 ml of supernatant, 2 ml of 3, 5- dinitrosalicylic acid solution was added. Put the solution for 5 minutes in water bath in boiling condition to let the colour developed. After colour development (dark red), 2 ml of distilled water was added to make the final volume to 5 ml. Absorbance was read at 540 nm against the supernatant from control soil sample. The values obtained were compared against a glucose standard curve prepared and these were reported as µg of glucose per gram of soil. The amylase activity was expressed as µg glucose g^{-1} soil $24h^{-1}$.

Standard curve

100 ml stock solution (1mg/ml) of glucose was prepared. A dilution series in the range of 100µg/ml, 200µ/ml, 400µg/ml, 600µg/ml, 800µg/ml, 1000µg were prepared. Measured the absorbance at 540 nm using UV-VIS spectrophotometer and the standard curve of concentration vs. absorbance was plotted.

Determination of soil cellulase activity

Chemicals and reagents

1. Toluene
2. Sorensen's buffer (pH 5.5,0.06M)
3. 1% Carboxymethyl cellulose solution
4. 3, 5-Dinitrosalicylic acid solution: Weighed 0.5gm of 3, 5-Dinitrosalicylic acid and dissolved in 20 ml of 2N sodium hydroxide and 50 ml distilled water. 30 gm

of Rochelle salt (Na, K- tartarate) was added to it and make up the volume to 100 ml with distilled water.

Procedure:

1 gm of soil sample (preserved at 4º C) was taken and 0.1 ml of toluene was added to it. Put it at room temperature for about 15 minutes. 2 ml Sorensen's buffer and 2 ml substrate solution (carboxymethyl cellulose solution) were added. Put it in incubator at 30º C for 24 hours. Control was prepared by taking water instead of substrate. After 24 hours, centrifuged the contents at 4000 rpm for 10 minutes. Pipetted out 1 ml of supernatant and add 2 ml of 3, 5-dinitrosalicylic acid solution. Solution was kept for 5 minutes in water bath in boiling condition to let the colour developed. After colour development (dark red), 2 ml of distilled water was added and final volume was made up to 5 ml. Absorbance was read at 540 nm against the supernatant from control soil sample. The values obtained were compared against a glucose standard curve prepared and these were reported as mg of glucose per gram of soil.

Standard curve

100 ml stock solution (1mg/ml) of glucose was prepared. A dilution series in the range of 100µg/ml, 200µ/ml, 400µg/ml, 600µg/ml, 800µg/ml, 1000µg were prepared. Measured the absorbance at 540 nm using UV-VIS spectrophotometer and the standard curve of concentration vs. absorbance was plotted.

Determination of soil urease activity

Chemicals and Reagents

1. Urea solution (0.2 M)
2. Tris-HCl buffer (pH 9.0)
3. Phenate solution
4. Potassium chloride-silver sulfate solution
5. Alkaline hypochlorite solution
6. Toluene
7. Standard ammonium solution

Procedure

To 5 g of soil about 0.2 ml of toluene and 9 ml of Tris- HCl buffer (pH 9.0) were added. The contents were shaken and then 1 ml of 0.2 M urea was added to it. Placed the stopper on the flask and incubated at 37ºC for 2 hours. After it the stopper was removed and the volume was raised to 50 ml with potassium chloride-silver sulphate solution. Centrifuged the content and 1 ml of phenate solution was added to 1 ml of supernatant followed by 1 ml of alkaline hypochlorite solution. Put. it at 37ºC for 5 minutes for colour development. 7 ml of distilled water was added and absorbance was measured at 625 nm. A control without urea was used with each sample. Urease activity was expressed as µg NH_3-N released/g soil/hr.

Standard curve

100 ml stock solution (5mg/100 ml) of Ammonium chloride was prepared. A dilution series in the range of 5µg/ml, 10µ/ml, 20µg/ml, 30µg/ml, 40µg/ml, 50µg were prepared. Measured the absorbance at 625 nm using UV-VIS spectrophotometer and the standard curve of concentration vs. absorbance was plotted.

Determination of dehydrogenase activity

Chemicals and Reagents

1. 2,3,5-Triphenyl Tetrazolium Chloride
2. Methanol
3. Triphenyl Formazan

Procedure

About 5 g of soil, 3% solution of 2,3,5 triphenyl tetrazolium chloride and 2.5 ml of distilled water were mixed and incubated at 37º C for 24 hours. After incubation 10 ml of methanol was added and shaken the contents for 1 minute. The suspension was filtered through Whatman No. 1 filter paper into a 50 ml volumetric flask. Further extraction was done with additional amount of ethanol until there was no reddish colour in methanol extract and the extract was raised to 50 ml with methanol. Measured the absorbance of supernatant at 485 nm with methanol as blank. Standard graph was prepared with 1,3,5 triphenyl tetrazolium formazon. Dehydrogenase activity was expressed as µg TPF g^{-1} soil 24 h^{-1} (Casida *et al*. 1964).

Standard curve

100 ml stock solution (1mg/1 ml) of 1, 3, 5 triphenyl tetrazolium formazon was prepared. A dilution series in the range of 100µg/ml, 200µ/ml, 400µg/ml, 600µg/ml, 800µg/ml, 1000µg were prepared. Measured the absorbance at 485 nm using UV-VIS spectrophotometer and the standard curve of concentration vs. absorbance was plotted.

Determination of Alkaline phosphatase activity

Chemicals and Reagents

1. p-nitrophenyl phosphate
2. p-nitro phenol solution (10mg/100 distilled water)
3. Acetate buffer (pH 11.0)
4. 0.5 M Sodium hydroxide solution
5. 0.5 M Calcium chloride solution

Procedure

To 1 g of soil 0.2 ml toluene, 3 ml acetate buffer (pH 11.0), and 1 ml p-nitrophenyl phosphate (1%) were added and incubated at 37º C for 1hour. After incubation 1ml of 0.5 M sodium hydroxide and 4 ml of 0.5 calcium chloride were added and mixed the content. Filtered the content through Whatman No.2. The supernatant containing yellow coloured p- nitro phenol was red at 420 nm against non p-NPP soil blank. The values obtained were compared against the standard curve. Alkaline phosphatase activity was expressed as µg of p-nitro phenol formed g^{-1}soil after 1 hour incubation at 37º C. (Makoi *et al*. 2010).

Standard curve

100 ml stock solution (0.1mg/1 ml) of p- nitro phenol was prepared. A dilution series in the range of 10µg/ml, 20µ/ml, 40µg/ml, 60µg/ml, 80µg/ml, 100µg were prepared. Measured the absorbance at 420 nm using UV-VIS spectrophotometer and the standard curve of concentration vs. absorbance was plotted.

Determination of acid phosphatase activity

Chemicals and Reagents

1. p-nitrophenyl phosphate solution
2. Acetate buffer (pH 6.5)
3. p-nitro phenol solution (10mg/100ml d/w.)
4. 0.5 M Sodium chloride solution
5. 0.5 M Calcium chloride solution

Procedure

To 1 g of soil 0.2 ml toluene, 3 ml acetate buffer (pH 6.5), and 1 ml p-nitrophenyl phosphate (1%) were added and incubated at 37 ºC for 1h. After incubation 1ml of 0.5 M sodium hydroxide and 4 ml of 0.5 M calcium chloride were added and mixed the content. Filtered the content through Whatman No.2. The supernatant containing yellow colored p- nitro phenol was read at 420 nm against non p-NPP soil blank. The values obtained were compared against the standard curve. Alkaline phosphatase activity was expressed as µg of p-nitro phenol formed /g soil after 1-hour incubation at 37º C. (Makoi *et al*. 2010).

Standard curve

100 ml stock solution (0.1mg/1 ml) of p- nitro phenol was prepared. A dilution series in the range of 10µg/ml, 20µ/ml, 40µg/ml, 60µg/ml, 80µg/ml, 100µg were prepared. Measured the absorbance at 420 nm using UV-VIS spectrophotometer and the standard curve of concentration vs. absorbance was plotted.

4.6. Physical and chemical characterization of soil

Determination of soil texture

Sand, silt and clay are the three particle sizes of mineral material found in soils and their proportionate amount determines the soil texture. The particle size for sand, silt and clay are 0.05-2.0 mm, 0.002-0.05 mm and less than 0.002 mm, respectively. To determine the percentage of sand, silt and clay, sieving method was used. Sieve analysis was made of a "nest" of sieves placed one above the other. The top sieve has 4.75 mm sized holes, below it are sieves with successively smaller holes 2.36 mm, 1.18 mm, 600 µ,300 µ.The weighed soil sample was placed in the upper most sieve and the sieve assembly was shaken until the soil particles pass through their respective sieves. The soil collected in each sieve was weighed and amount of sand, silt and clay was noted.

Determination of soil pH

The soil was taken in a beaker and distilled water was added to it in the ratio 1:5 (w/v). Mixed the slurry by continuous stirring with magnetic stirrer for about 10

minutes. Allowed the slurry to stand for 15 minutes. The pH of slurry was measured by immersing the electrode and the value read on a digital electronic pH meter.

Determination of conductivity

The soil was taken in a beaker and distilled water was added to it in the ratio 1:5 (w/v). This slurry was mixed by continuous stirring with magnetic stirrer for about 10 minutes and then allowed to stand for 15 minutes. The conductivity of the slurry was measured by immersing the electrode of digital conductivity meter in the slurry and value was measured as µs.

Determination of organic carbon and organic matter

Chemicals and Reagents

1. Potassium dichromate solution (1N)
2. Ferrous sulphate (0.5N)
3. Phosphoric acid
4. Diphenylamine indicator
5. Sulphuric acid

Procedure

Organic carbon and organic matter was estimated by rapid titration method (Walkley and Black, 1934). 1 g sieved soil was taken in dry 500 ml conical flask and 10 ml of $K_2Cr_2O_7$ was added and followed by addition of 20 ml of conc. H_2SO_4. The contents of flask shaken by hand for 1 minute and kept aside for 30 minutes. Then 200 ml of distilled water was added followed by addition of 10 ml of phosphoric acid and 1 ml of diphenylamine indicator. The contents of flask turned blue and titrated against 0.5N ferrous sulphate solution until the colour changed to green.

Calculations:

One ml of 1N $K_2Cr_2O_7$ is equivalent to 3 mg of carbon. The amount of carbon oxidized, expressed as percentage of soil is given by:

% Organic Carbon = Titre value (ml) ×0.003 ×100/Weight of soil taken (g)

Where,

Titre value = total volume of $K_2Cr_2O_7$ added - 1/2 volume of N/2 $FeSO_4$ used

Organic matter is a function of percent organic carbon and calculated as:

% Organic matter = % Organic Carbon×1.724

Determination of available nitrogen

Chemicals and Reagents

1. Potassium permanganate solution (0.32%)
2. Sodium hydroxide solution (2.5%)
3. Sulphuric acid (0.02N)
4. Sodium hydroxide solution (N/50)
5. Methyl red indicator

Procedure

Available Nitrogen from soil was estimated by alkaline potassium permanganate method (Association of official agricultural chemists, AOAC, 1960). Twenty gram of soil sample was taken in 500 ml Kjeldhal distillation flask. To it was added 20 ml of distilled water followed by 100 ml of 0.32% $KMnO_4$ and 100 ml of 2.5 % sodium hydroxide, and fitted in distillation apparatus. The end of delivery tube was immersed in a 250 ml of conical flask containing 20 ml of N/50 sulphuric acid and 2-3 drops of methyl red indicator. The distillation flask was heated and the produced ammonia gas was collected in conical flask until the volume in the flask becomes 100 ml. The excess of sulphuric acid in conical flask was titrared against N/50 sodium hydroxide till the colour changed from pink to yellow.

Calculations:

Weight of soil taken = 20 g

Volume of 0.02N H_2SO_4 taken = 20 ml

Volume of 0.02N NaOH taken = x ml

Volume of 0.02N acid used for absorbing NH_3 = (20-X) ml

Available Nitrogen = (20-x) × 20 Kg /ha

Determination of available phosphorus

Chemicals and Reagents

1. Sodium bicarbonate ($NaHCO_3$) solution, N/2
2. Ammonium molybdate solution
3. p-nitrophenol indicator solution (0.5) w/v
4. Sulphuric acid
5. Standard Phosphorus solution
6. Reagent A: Dissolved 12g of ammonium molybdate in 250 ml of distilled water and 0.2906 g of antimony potassium tartarate in 100 ml of distilled water,

added these two solution in 1000 ml of 2.5M H_2S0_4**,** mix thoroughly and make up to 2000 ml with distilled water.

7. Reagent B: Dissolved 1.056g of ascorbic acid in 200 ml of Reagent A

Procedure

Available phosphorous was determined as per the procedure of Olsen *et al*. (1954). 25 ml of 0.5M $NaHCO_3$ was added to 2.5 g soils taken in 100 ml conical flask and agitated at 130 rpm for 30 min on an electric shaker. The mixture was filtered through quantitative filter paper (grade equivalent to Whatman 42). 2 ml of aliquot was transferred to 100 ml beaker followed by addition of 0.2 ml of 2.5M H_2SO_4 and 3 ml of distilled water. To this mixture, add 3.1 ml of distilled water and 1.6 ml of Reagent. Absorbance of the samples were read spectrophotometerically at 882 nm and compared with standard plot.

Standard curve

0.2, 5, 10, 15 and 20 ml of standard phosphorus solution KH_2PO_4 were taken in 6 different 50 ml volumetric flasks followed by addition of 10 ml of 0.5 M $NaHCO_3$ and 1.0ml 2.5 M H_2SO_4 to each flask. 8 ml of reagent B was added to each and volume was adjusted to 50 ml with distilled water. The phosphorous concentration of these solution will be 0.04, 0.1, 0.2 0.30 and 0.40 mg/L respectively. A standard curve was plotted showing relationship between concentrations of phosphorous and absorbance.

Calculation

P in soil (mg/kg) = P in extract (mg/L) x 20 (the standard soil to solution ratio)

4.7. Statistical analysis

There were at least three replicates of each parameter or experiment. The data are expressed as the means ± the standard deviation of the means. Significance among the data was determined by Analysis of variance (ANOVA).

Chapter 4 MATERIALS & METHODOLOGY

Chapter 5
Results & Discussion

5.1. Results

5.1.1 Soil sampling

Soil samples were collected from surface (0-20 cm depth) of agricultural fields near Bathinda, District. Where Bt cotton (RHC134) and non Bt cotton variety had been continuously cultivated for last two years. Soil samples were collected after harvesting of cotton crop. A control sample was collected from the adjoining waste land where no crop was grown from last many years. Collected soil samples were ground and sieved through 2 mm sieve and stored at -20ºC before analysis. After it the soil samples for enzymatic assay were kept at 4ºC and for physical-chemical analysis kept at room temperature before a week for experiment setup.

5.1.2 Enzyme activities in soil samples

Enzyme activities such as amylase, cellulase, urease, dehydrogenase, alkaline and acid phosphatases in different soil samples studied after harvesting showed differences between Bt and non Bt cotton field soil samples. And most of the changes were statistically significant ($P<0.05$).

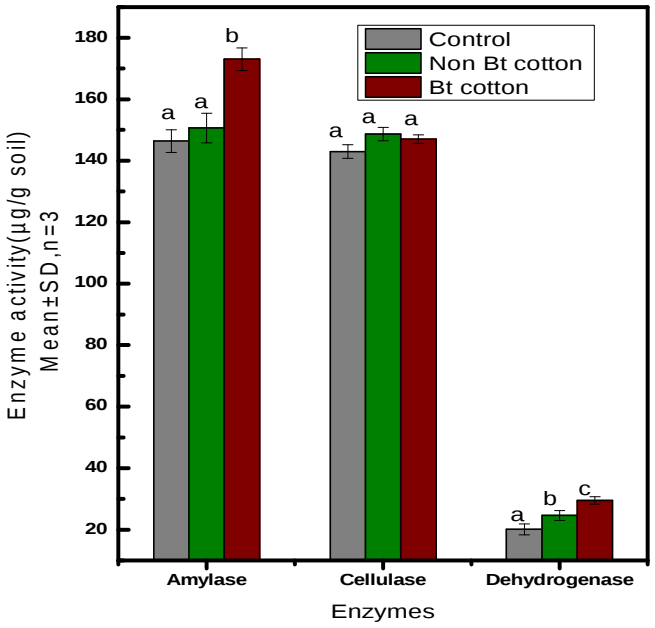
5.1.2.1 Amylase activity

It is clear from the Fig 1.1 and 1.2 that amylase activity was enhanced in Bt cotton as compared to non Bt cotton soil sample and control sample. A significant increase up to 18 % has been observed in case of Bt cotton soil while in non Bt soil sample it was only 3% as compare to the control. Similar results were reported by Sun *et al.* (2007) and Wu *et al.* (2004). They observed increased activity of soil enzymes in GM crop soil samples as compared to control soil samples.

5.1.2.2. Cellulase activity

Cellulase activity was not significantly affected in both Bt cotton and non Bt cotton soil samples as compared to the control sample (fig.1.1, 1.2). It was slightly increased in both Bt cotton soil sample and non Bt cotton as compared control. It was increased in non Bt (3%) and in Bt (2%). Sun *et al.* (2007) reported similar result that is the addition of biomass of cotton to the soil stimulated cellulase activity.

Figure 1: Amylase, cellulase and dehydrogenase activities in soil samples


Control
Non Bt cotton
Bt cotton
Enzyme activity(µg/g soil)
Mean±SD,n=3
180
160
140
120
100
80
60
40
20
a
a
b
a
a
a
a
b
c
Amylase
Cellulase
Dehydrogenase
Enzymes

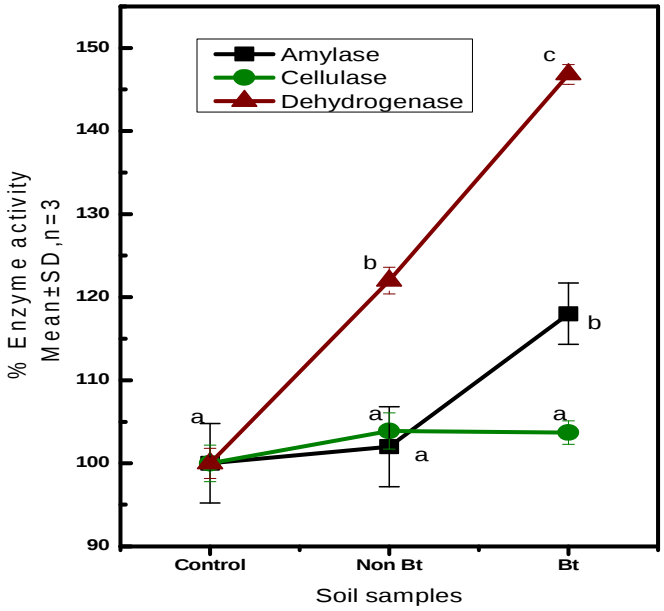


Figure 1.1: Bar graph showing enzyme activities in µg/g soil/h in soil samples

Different alphabets along each line represents significant differences over control at P ≤ 0.05 applying Tukey's test.

Figure 1.2: Line graph showing percent increase in enzyme activities in different soil samples as compared to control.

5.1.2.3. Dehydrogenases Activity

Dehydrogenase activity was enhanced in both tton (22%) soil samples as compared to the control s ient in dehydrogenase activity in Bt cotton soil samp tton soil sample (fig.1.1, 1.2). The differences were s this regards different reports has been observed sim ilar results Wu et *al.* (2004) and Mina *et al*. (2011) observed increases in the activity of dehydrogenase.

5.1.2.4. Urease activity

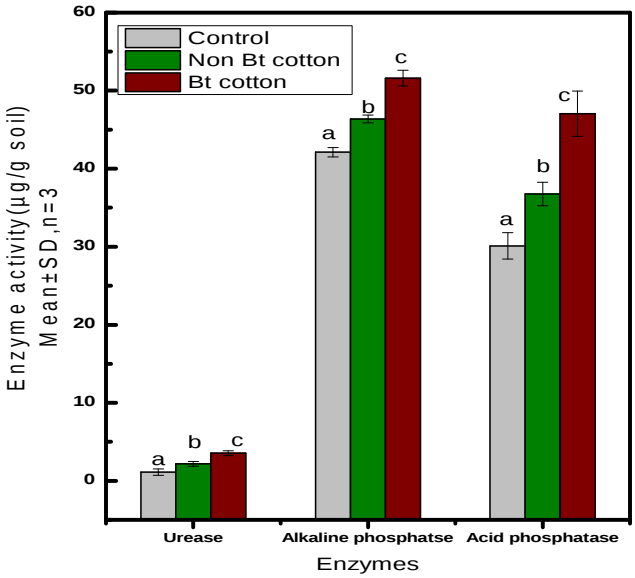
It is clear from the fig. 2.1 and 2.2 that urease activity was enhanced in both Bt cotton and non Bt cotton soil samples as compared to the control sample. A significant increase up to 3.2 times has been observed in case of Bt cotton soil while in non Bt

soil sample it was 1.97 times as compare to the control. Same results were reported by Sun *et al*. (2007) and Falih and Wainwright, 1996.

5.1.2.5. Alkaline phosphatase and acid phosphatase activity

Alkaline phosphatase and acid phosphatase activity was enhanced in both Bt cotton and non Bt cotton soil samples as compared to the control sample. There was also enhancement in activity of these enzymes in Bt cotton soil samples as compared to the non Bt cotton soil sample (Fig.2.1, 2.2). Alkaline phosphatase activity was increased up to 10% in non Bt cotton soil samples and up to 22% in Bt cotton soil samples as compared to control. Acid phosphatase activity was enhanced up to 22% in non Bt soil samples and up to 56% in Bt cotton soil samples as compared to control soil sample. These differences were statistically significant ($p<0.05$). Results were also supported by Wu *et al*. (2004) and Sun *et al*. (2007) study, they observed increased activities of phosphatases by the addition of biomass of Bt rice straw and Bt cotton to the soil.

Figure 2: Urease, alkaline phosphatase and acid phosphatase activities in soil sample.


Control
Non Bt cotton
Bt cotton
Enzyme activity(µg/g soil)
Mean±SD,n=3
60
50
40
30
20
10
0
a
b
c
a
b
c
a
b
c
Urease
Alkaline phosphatse
Acid phosphatase
Enzymes

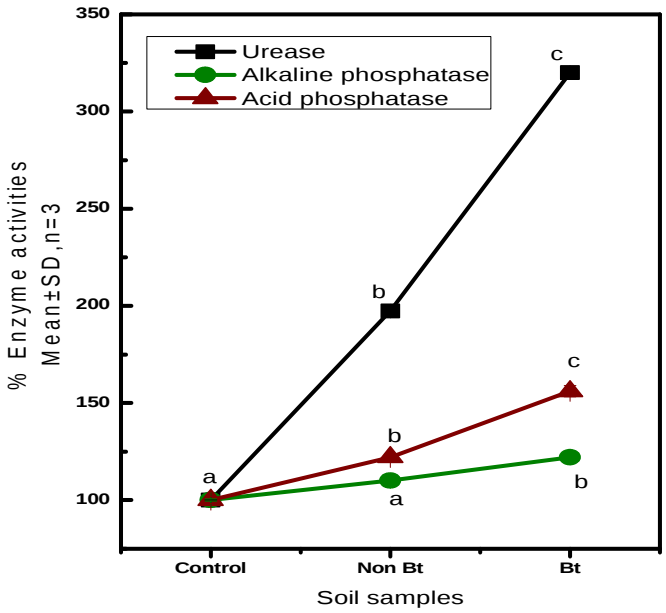


Figure 2.1: Bar graph showing enzyme activities in µg/g soil/h in soil samples

Different alphabets along each line represents significant differences over control at P ≤ 0.05 applying Tukey's test.

Figure 2.2: Line graph showing percent increase in enzyme activities in different soil samples as compared to control

Bt cotton and Non Bt cotton tissues had significant rent enzyme activities. Soil micro-organisms are one of th nes (Nannipieri *et al*., 1983). The addition of Bt cotto for supplying higher content of organic product, and ably responsible for the increase in amylase, ure line phosphatase and acid phosphatase activities of soil.

5.1.3 Physical and chemical characterization of soil samples

Different physico-chemical parameters of soil samples such as pH, conductivity, percent organic carbon, percent organic matter, available nitrogen and available phosphorous were studied. Soil texture of samples was sandy loam to silt.

5.1.3.1. pH and Conductivity

There was no variation in pH between Bt, non Bt cotton soil samples and control (Fig.3.1, 3.2). Significant increase in conductivity was observed in Bt cotton soil samples i.e. up to 89% as compared non Bt cotton samples. This difference was statistically significant ($p<0.05$).

5.1.3.2. Percent organic carbon and organic matter

It is clear from the figure 4.1 and 4.2 that percent organic carbon and organic matter was higher in both Bt cotton and non Bt cotton soil samples as compared to the control sample. Percent organic carbon and organic matter content in Bt cotton soil samples was higher as compared to the non Bt cotton soil sample. In Bt cotton soil samples percent organic carbon was increase up to 54% and organic matter 53%, while in non bt soil sample it was only up to 7 % and 6% respectively. Difference was statistically significant ($p<0.05$). Similar results were reported by Liu *et al*. (2010) they observed higher organic matter in GM maize as compared to non GM maize control.

Figure 3: pH and conductivity in different soil samples

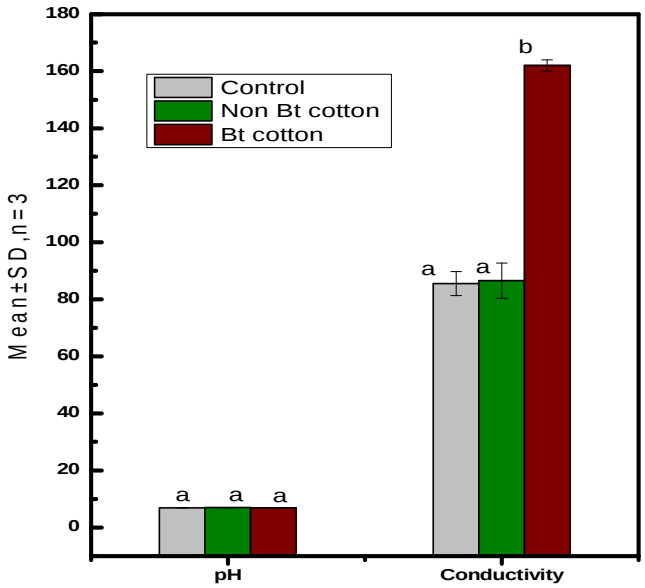


Figure 3.1: Bar graph showing pH and conductivity (µs) in soil samples

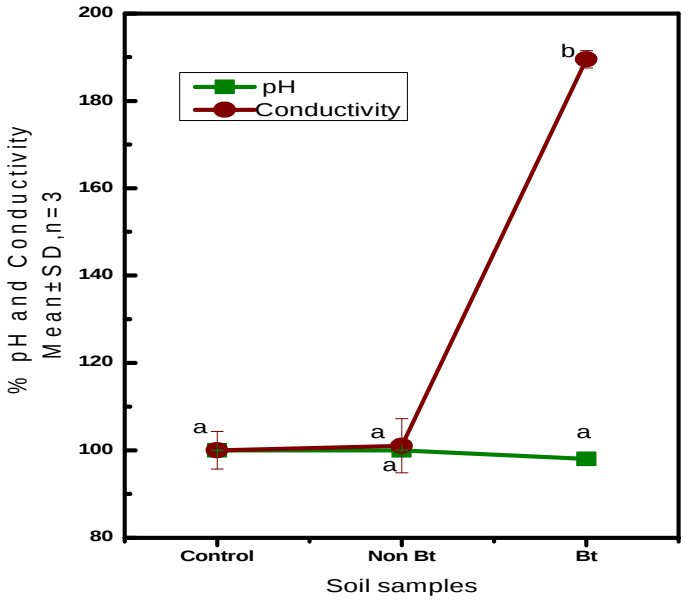
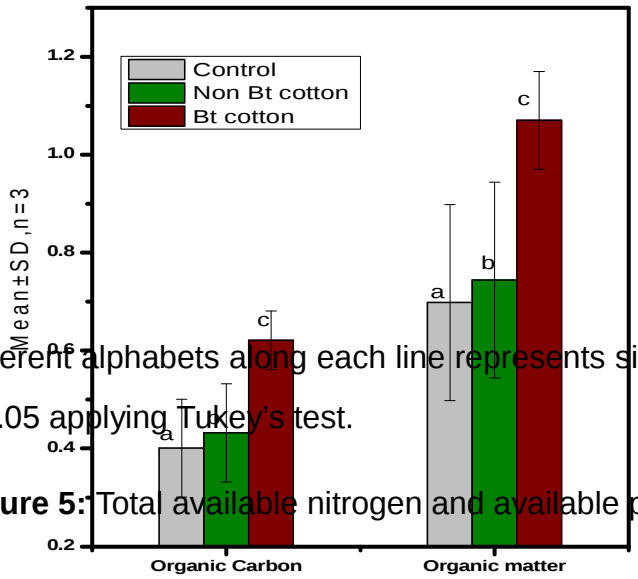


Figure 3.2: Line graph showing percent increase in pH and conductivity in soil samples as compared to control

Different alphabets along each line represents significant differences over control at P ≤ 0.05 applying Tukey's test.

Figure 4: Percent organic carbon and organic matter content in the soil samples

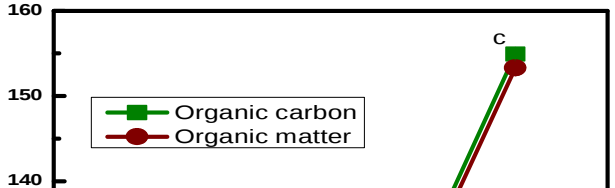
Figure 4.1: Bar graph showing percent organic carbon and organic matter content in soil samples.



Different alphabets along each line represents significant differences over control at P ≤ 0.05 applying Tukey's test.

Figure 5: Total available nitrogen and available ph

Figure 4.2: Line graph showing percent increase in organic carbon and organic matter content in soil samples as compare to control.



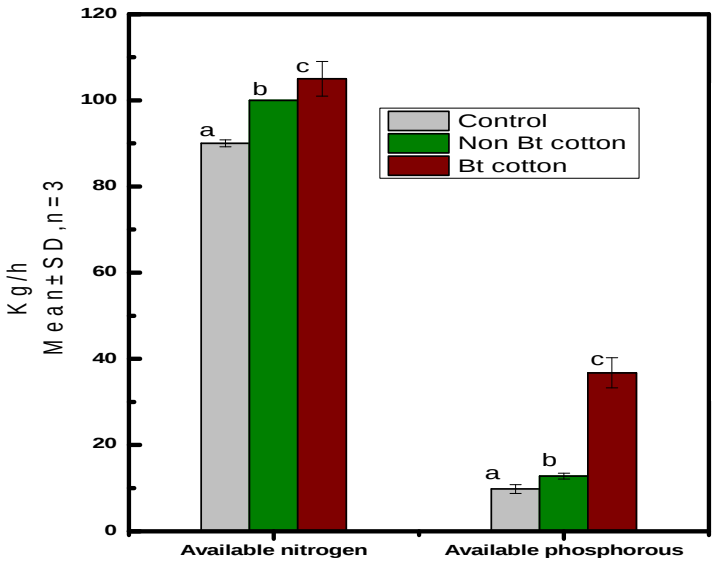


Figure 5.1: Bar graph showing total available nitrogen and available phosphorous content in Kg/h in soil samples.

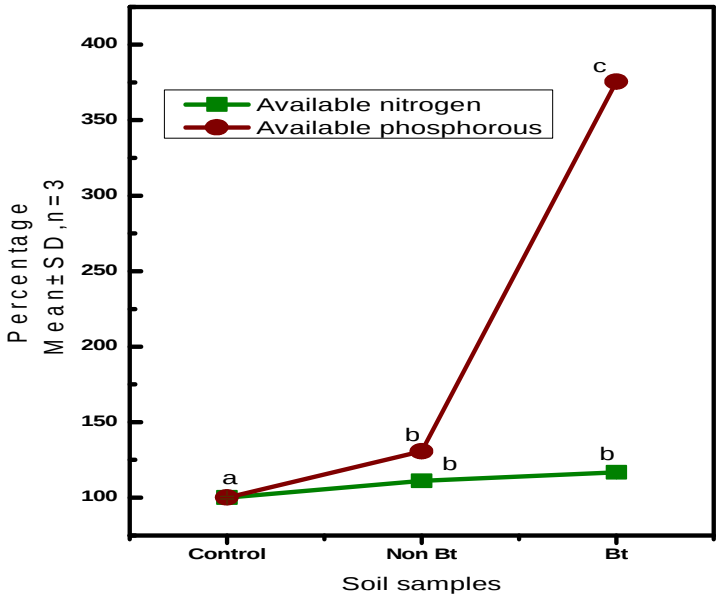


Figure 5.2: Line graph showing percent increase in total available nitrogen and available phosphorous content in soil samples as compared to control

Different alphabets along each line represents significant differences over control at P ≤ 0.05 applying Tukey's test.

5.1.3.3. Available nitrogen and available phosphorous

Available nitrogen and available phosphorous contents also follow similar trend *i.e.* higher in Bt cotton and non Bt cotton soil samples as compared to the control sample. There was also more available nitrogen and available phosphorous content in Bt cotton soil samples as compared to the non Bt cotton soil sample (Fig.5.1, 5.2). Available nitrogen was increased in non Bt (11%) and in Bt (16%) as compared to control soil sample. Available phosphorous was increased in non Bt (1.3 times and in Bt (1.3 times as compared to control soil sample. These differences were statistically significant ($p<0.05$). Similarly the enhancement of available nitrogen reported by Liu *et al*. (2010) in GM maize.

Conclusion and future directions

Continuous cultivation of Bt cotton could lead to accumulation of Bt toxin in the soil. This could cause damage to beneficial micro flora of the soil, necessary for plant and soil health, litter decomposition and nutrient cycling. For evaluating soil health we studied the parameters like enzyme activities and physical-chemical parameters between Bt, non Bt cotton soil and control soil after harvesting. Enzyme activity is the parameter which gives earlier indication about the effect of any practice on soil health. The result of the study discussed can be summarized as follows:

- Statistically significant enhancement in activities of enzymes like amylase, urease, dehydrogenase, alkaline phosphatase and acid phosphatase were observed in soil samples from cotton fields as compared to control soil.
- Significant increase in enzyme activities like amylase, urease, dehydrogenase, alkaline phosphatase and acid phosphatase were observed in Bt cotton soil samples as compared to non Bt cotton soil samples.
- No significant difference observed in cellulase activity between Bt and non Bt cotton soil sample.
- Statistically significant higher organic carbon, organic matter, available nitrogen and available phosphorous content were found in Bt cotton soil samples as compared to non Bt cotton soil samples.

By concluding above results, there were positive effects of Bt cotton cultivation in case of surface soil. But to evaluate long term effects of continuous cultivation of Bt cotton, more data should be required. The release of Bt toxin is mostly governed by temperature, humidity and other environmental conditions. So we cannot say that Bt cotton is as much as effective to Indian environment as in Australia and other cold countries. Further study will be needed to study: (1) The effect of Bt cotton on soil biota in Indian climate context and (2) To evaluate the possible risk potential of cultivating GM crops before releasing more GM crops to be cultivated.

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Summary

The present study focuses on the impacts of Bt (*Bacillus thuriengiensis*) cotton crop cultivation on soil health. The experimental work includes the study of soil enzyme activities and physico-chemical parameters of soil from cotton fields of Bhatinda, District. Where Bt cotton (RHC134) and non Bt cotton variety had been continuously cultivated for last two years. Soil samples were collected after harvesting of cotton crop. A control sample was collected from the adjoining waste land where no crop was grown from last many years.

Due to climate change and use of agricultural land for multiple purposes, it is a major question of how to feed or fulfil the needs of growing population of the world in the coming years. The genetic modification of plants is in highlights for its promising use in sustainable development of agricultural system. New transgenic crop plants with high yield and improved resistance against pests, pathogens, parasites etc. are the outcome of this technology. Bacillus *thuringiensis* (Bt) cotton is the only genetically modified crop which was introduced in India in 2002. The area under cultivation of genetically modified crops is increasing day by day. As a result, the bio safety of these crops has been a major concern in recent years, and many studies related to effects of Bt cotton and other GM crops on soil health have been conducted in other parts of world like US, Australia, China etc. (Men *et al*., 2003; Bai *et al*. 2003; Li *et al*., 2002; Liu *et al*., 2002; Zhang *et al*., 2000). Some studies revealed that transgenic Bt cotton has no harmful effects on soil health may even have beneficial effects, while other studies have reported some adverse effects (Cui and Xia 2000; Tan *et al*., 2002). Therefore, a complete understanding about the impacts of genetically modified crops on soil health as well as other bio safety aspects would contribute to future agricultural production in an environmentally sound and sustainable manner.

Our present study has provided information concerning the effects of growing Bt cotton vs. non Bt cotton on soil health. Growing Bt cotton instead of conventional cotton appeared affect positively soil health.

To test the effect of Bt cotton, we measure the activities of different soil enzymes like amylase, cellulase, urease, dehydrogenase, alkaline phosphatase and acid phosphatase, spectrophotometrically, based on the substrate degrading ability of

enzymes. Soil enzymes are most eligible candidate among the biological indicators. As these play an important role in catalyzing various reactions necessary in organic matter decomposition and nutrient cycling. They are involved in energy transfer, environmental quality and crop productivity processes (Dick, 1994; Tabatabai, 1994). Soil enzyme activities are greatly affected by organic matter content of the soil and often are used as indices of microbial activity and soil fertility (Dick and Tabatabai, 1992; Kumar *et al*., 1992). However, the transgenic Bt cotton had positive effects on the tested enzyme activities in the soil. Statistically significant enhancement in activities of different enzymes like amylase, urease, dehydrogenase, alkaline phosphatase and acid phosphatase were observed in soil samples from cotton fields. There was significant increase in different enzyme activities in Bt cotton soil samples as compared to non Bt cotton soil samples. While there was no difference observed in cellulase activity between Bt and non Bt cotton soil sample.

The effects of genetically modified cotton expressing the *Bacillus thuringiensis* toxins on soil chemistry (organic carbon and matter, N and P), compared with non-GM and controls, were assessed. Significant effects of using Bt cotton instead of conventional cotton were found. Statistically significant higher organic carbon, organic matter, available nitrogen and available phosphorous content were found in Bt cotton soil samples as compared to non Bt cotton soil samples.

Present study showed direct relationship between enzyme activity and soil nutrient availability. Amylase, cellulase and urease activity are related with more organic carbon, organic matter and available nitrogen content in the Bt cotton soil. In the same way, more content of available phosphorous was result of increased activities of alkaline phosphatase and acid phosphatase in the soil.

Even significant effects appeared to be small. No general conclusion can be drawn based on our results; it needs more study on the topic regarding long term cultivation of GM crops.

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