Utilization Certificate

Innovation Project 2015-16

SHC-306

Project Title: Application of Biocontrol Agents and Herbal Oils on Wheat Crop Against Fungal Disease (*Fusarium*)

Audited Financial Statement under Innovation Project scheme

College: Shivaji College

Project Investigators: Dr. Promila Mathur, Dr. Seema Talwar and Dr. Nupur Mondal

Grant	Amount Sanctioned	Utilized	Balance (
Equipments/Consumables (shifted from equipments/consumables)	Rs.1,00,000+55,000	Rs. 1,53,875	Rs. 1,125
Travel (shifted to equipments/consumables	Rs. 55,000	.....	.....
Stipend	Rs. 1,20,000	Rs. 1,20,000	.....
Honorarium	Rs. 25,000	Rs. 25,000	.....
Stationery	Rs. 20,000	Rs.17,737	Rs. 2,263
Contingency	Rs. 30,000	Rs. 26,727	Rs. 3,273
Total amount utilized Rs. (In figures and words)	Rs. 3,43,339 (Three Lakhs Forty Three Thousand Three Hundred Thirty Nine)		
Amount remaining Rs. (In figures and words)	Rs. 6,661 (Six Thousand Six Hundred Sixty One)		

Certified that out of Rs. 3,50,000 (Three Lakhs and fifty thousand) sanctioned to Innovation Project Code **SHC-306**, Rs. **3,43,339** (Three Lakhs Forty Three Thousand Three Hundred Thirty Nine) has been utilized during the period of the project. The remaining amount of Rs. **6,661** (Six Thousand Six Hundred Sixty One) is being returned back to the University.

Signature of Project Investigators

Dr. Promila Mathur P. Mathur

Dr. Seema Talwar S Talwar

Dr. Nupur Mondal N Mondal

Signature of Principal

04.11.16

VIPUL CHAWAN & ASSOCIATES ★ Chartered Accountants ★ CA M. No. 518146 NEW DELHI

Financial Audit Clearance
and Stamp of Chartered Accountant

University of Delhi

RC/2015/9435 31 August, 2015

The Principal,
Shivaji College
Ring Road, Raja Garden,
New Delhi-27

Subject: - **Innovation Projects 2015-16**

Dear Principal,

The University of Delhi is pleased to announce the third round of the undergraduate research initiative in colleges, Innovation Projects 2015-16. You will be glad to know that the following project submitted by your college has been selected for award

Project Code: SHC 306
Project Title: Application Of Biocontrol Agents And Herbal Oils On Wheat Crop Against Powdery Mildew Disease

The distribution of grant under different budget heads as below:

Sr. No.	Budget Head	Amount
1.	Equipment/Consumables	Rs 100,000/-
2.	Stipends	Rs. 1,20,000/- (1000x10x12)
3.	Travel	Rs 55,000/-
4.	Honorarium	Rs 25,000/-
5.	Stationery/Printing	Rs 20,000/
6.	Contingency	Rs 30,000/-
	Total	Rs 350,000/-
Rs 3.5 lakhs (Rupees three lakhs fifty thousand only)		
Amount to be released in first phase by Finance Branch- Rs 2,50,000/		

Budget head No. 1 and half of the remaining grant will be released as the first instalment. The second and final instalment will be released after submission of half-yearly report (by 15 February 2016), satisfactory review and recommendation of release of the second instalment.
Please refer to the detailed guidelines for implementation of the project. Any queries may be addressed to- innovationprojects1516@gmail.com.

With best wishes,

Yours sincerely,

Prof. Malashri Lal

Application of Biocontrol Agents and Herbal Oils on Wheat Crop Against Fungal Disease (*Fusarium*)

Project Proposal

Delhi University Innovation Project, 2015

Shivaji College
University of Delhi
Raja Garden
New Delhi -110027

Submitted by

1. **Dr. Promila Mathur**
2. **Dr. Seema Talwar**
3. **Dr. Nupur Mondal**

Mentor

Dr. Sujata Bhardwaj
Deptt. Of Biology
Bhaskaracharya College of Applied Sciences
University of Delhi,

1. PROJECT CODE: SHC 306

2. PROJECT TITLE : Application of Biocontrol Agent and Herbal Oils on Wheat Crop Against Fungal Disease (*Fusarium*)

3. NAME OF COLLEGE/INSTITUTION: Shivaji College

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University of Delhi

Certificate of Originality

This is to certify that the research work carried out and the final report submitted By the Project Investigators and the students of Innovation Project having Project code: **SHC 306**and title **"Application of Biocontrol Agents and Herbal Oils on Wheat Crop Against Fungal Disease (*Fusarium*)"**Of College/ Institute **Shivaji College** is original. Any plagiarism/academic dishonesty reported at any stage will be our responsibility.

Signatures of the all PIs

1. Dr. PromilaMathur
2. Dr. Seema Talwar
3. Dr. NupurMondal

Mentor

Dr. Sujata Bhardwaj
Associate Professor
Bhaskaracharya College of Applied Science
University of Delhi, Delhi

Utilization Certificate

Innovation Project 2015-16

SHC-306

Project Title: Application of Biocontrol Agents and Herbal Oils on Wheat Crop Against Fungal Disease (*Fusarium*)

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Signature of Project Investigators

Dr. Promila Mathur

Dr. Seema Talwar

Dr. Nupur Mondal

Financial Audit Clearance
and Stamp of Chartered Accountant

Signature of Principal

Final Report

1. **Project Title:** Application of Biocontrol Agent and Herbal Oils on Wheat Crop Against Fungal Disease (*Fusarium*)
2. **Project Code**: SHC 306
3. **Abstract**:

Medicinal plants produce high amount of active principles, which have been used in pharmaceutical industry for their antimicrobial property. These active principles are basically secondary metabolites which are the by-products of primary metabolic pathways. These are viewed as potential source of natural drugs, antibiotics, insecticides and herbicides. The medicinal property of most of the plants is associated with the production of essential oils. Essential oils and their extracts have the ability to control various microorganisms (bacteria, fungus, and virus) which cause infection to plants, humans and also spoil the food. These are generally aromatic organic compounds and broadly include phenolics, terpenes, steroids and alkaloids. The active antimicrobial compounds of essential oils are generally terpenes, which are phenolic in nature. These phenolic compounds are responsible for the inhibition of various fungal pathogens.

Fungus like *Fusarium* has been recognized to be the major plant pathogen all over the world. This fungal plant pathogen results into a great loss to our agriculture every year. Essential oils and their extracts have less toxic side effects than chemical insecticides and pesticides. The pharmacological value of the essential oils is increasing day by day due to their potential role as biocontrol agents, non-phytotoxic compounds and potentially effective against several microorganisms.

Present study was conducted to find the antifungal activity of herbal oils of Clove (*Syzygium aromaticum),* Neem (*Azadirachta indica),* and Peppermint against the fungal pathogen *Fusarium graminearum* which causes head blight disease on wheat. In addition to this different concentration of yeast (*Saccharomyces cereviseae*) (0.1%, 0.2%, 0.3%, 0.4% and 0.5%) were also applied as bio-control agent to control the fungus.Among the clove, neem and peppermint oil, clove oil is considered to be the best as it showed the highest antifungal activity at 1μLml-1; on this concentration clove essential oil caused

complete growth inhibition of *F. graminearum*, whereas among the different concentration of yeast, 0.5% was considered to be the best as at this concentration no fungal growth was observed.

4. Introduction:

Fusarium head blight of wheat (FHB), also called head scab, is caused mainly by the fungus *Fusarium graminearum* (also known as *Gibberella zeae*). This disease periodically causes significant yield loss and reduced grain quality. *F. graminearum* also produces mycotoxins, deoxynivalenol (DON), also known as vomitoxin that are toxic to humans and livestock. Seeds infected with *F. graminearum* have poor germination, resulting in slow emergence, and can be affected by seedling blight disease. Infected seedlings appear reddish-brown to brown.

Plant essential oils are potential source of antimicrobials of natural origin. Essential oils and extracts obtained from many plants have recently gained a great popularity and scientific interest. Consumer demand for natural preservatives has increased, whereas the safety aspect of chemical additives has been questioned. The plant oil has been reported to have antibacterial, antifungal, antiviral, antiparasitic and antidermatophytic properties. It is now considered as a valuable source of natural products for development of medicines against various diseases and also for the development of industrial products.

5. Research problem/hypothesis/objectives

India is an agricultural country. The Indian economy is basically dependent on agriculture. In spite of economic development and industrialization, agriculture is the backbone of the Indian economy. Nearly two-third of its population depends directly on agriculture for its livelihood. In spite of the fact that large areas in India, the productivity of agriculture is very low. Wheat is the most important food-grain of India and is the staple food of millions of Indians, particularly in the northern and north-western parts of the country. The time of sowing and harvesting differs in different regions due to different climatic variations.The temperature should be low at the time of sowing but as the harvesting time approaches higher temperatures are required for proper ripening of the crop. But sudden decrease in temperature at the time of maturity, frost at flowering time and hail storm at the time of ripening can cause heavy damage to the wheat crop.It is widely accepted that a number of diseases caused by fungi,

bacteria and viruses results in higher yield losses. Loss of crop from fungal plant pathogen may result in hunger and starvation, especially in developing countries where access to disease-control methods is limited and annual losses of 30 to 50 percent are common for major crops.

Head blight is a one of the destructive diseases of wheat, which is caused by a fungus *Fusarium graminearum.* To prevent this devastation in wheat, growers often rely on chemical insecticides and pesticides. These chemicals, no doubt has helped in increasing yields per hectare as well as total production but it also resulted in environmental pollution, ill health to biotic community, and hazardous to the ecological systems. Therefore, the biological method of plant disease management seems to be a better alternative to these chemicals. The application of biological controls using antagonistic microorganisms has proved to be successful for controlling various plant diseases. Biological control promises to be a useful alternative approach in the control of plant pathogens in the sustainable agriculture system. These biological pesticides have been proved to be a potential source of eco-friendly and safe antimicrobial agents useful in plant protection. Similarly, herbal oils (peppermint oil, tea extract, mint oil, eucalyptus oil, basil, chenopodium oil, clove oil, neem oil) are also environmental safe, so they are more easily acceptable and also less hazardous to plants and animals. Keeping this in mind, the present investigation aims to study the effect of fungi (yeast) and herbal oils (clove oil, neem oil, peppermint oil) against the head blight,a common disease is found in North Western Plain Zone.

OBJECTIVES:

1. To compare the per cent seed germination in control and *Fusarium* treated plants treatments.
2. To examine the antifungal activity of clove oil, neem oil and peppermint oil against the fungal pathogen (*Fusarium*).
3. To study the antagonistic ability (fungicidal) of *Saccharomyces cerevisiae*.
4. To protect the wheat crop against *Fusarium* by using essential oils and yeast
5. To compare the morphological and bio-chemical aspects of differently treated plants
6. To statistically analyze data.

6. Methodology Techniques/Sampling /Tools/Materials:

6.1 Material:

Seeds of wheat were procured from National Seed Centre, Indian Agriculture Research Institute, New Delhi.Culture *(Fusarium graminearum*)was obtained from Indian Type Culture Collection, Indian Agriculture Research Institute, New Delhi.This culture was maintained on Potato Dextrose Agar and stored as active culture at 4^{o}C. It was sub-cultured after every 15 days to revive it for longer duration.

6.2 Per cent Seed Germination *in vitro*

Seed were soaked in the water (control) and *Fusarium graminearum*24 hours before sowing *in vitro.* After 24 hours per cent seed germination was calculated by the formulae:

Per cent germination (%) = Seed germinated /Total no. of seed x100

6.3 Screening of antifungal activity

Effect of the herbal oil was assessed by agar diffusion plate method. Different concentrations (0.1, 0.2, 0.5, 1, 2 and 5 µl/ml) of herbal oils were incorporated into PDA medium at the time of pouring in sterilized Petri dishes. Inoculum was taken from 7 days old culture and placed in the middle of a PDA plate. Petri plates were sealed with parafilm and incubated at 28±2°C for 7 days.Three replicates of each treatment were kept in incubator. The radial growth of colonies was measured with the help of a scale. For yeast, commercially active dry yeast was used. It was soaked in the water for 20-25 minutes and then the different concentrations (0.1%, 0.2%, 0.3%, 0.4% and 0.5%) were used.

Percentage of inhibition of colony growth was calculated by formula given by Djordjevic et al. (2013).

Per cent inhibition (I%)= gc-gt /gc x100

Where gc is the growth of mycelium in control plates, gt is the growth of mycelium in treated plates.

6.4 Estimation of chlorophyll (Hiscox and Israelstam, 1979)

Fresh leaves of plants were cut into small pieces.Leaf tissue (0.1g) was taken in a test tube containing seven ml dimethyl sulphoxide (DMSO) and incubated at 65^{0}C for one hour. To this aliquot, three ml DMSO was added to make total volume to ten ml. Absorbance was taken at 480 nm.

6.5 Estimation of protein (Bradford, 1976)

Plant material (grain, 0.5g) was taken and homogenized in 1.5ml phosphate buffer (0.1M Phosphate buffer pH 7.2), homogenate was filtered. 10% Tri-chloroacetic acid (0.5ml), was added in 0.5ml supernatant, followed by centrifugation at 4^{o}C; 3300 rpm for 10minutes. Pellet formed was washed with water 2-3 times and dissolved in 10ml 0.1N NaOH. Aliquot 0.1ml was taken and volume made to 1ml. Bradford reagent (5ml) was added and vortexed. The absorbance was observed at 595nm.

6.6 Estimation of carbohydrates (Hedge and Hofreiter, 1962)

Weigh 100mg of the sample. Hydrolyse it in boiling water bath for 3 hours with 5 ml. of 2.5N HCl. Neutralise it with solid sodium carbonate until effervescence ceases. Make up it by 1000ml and centrifuge. Take the 1ml supernatant and add 4ml of anthranone reagent. Heat for 8 minutes in water bath. Cool rapidly and take the optical density at 630nm.

7. Result (main text, tables with titles, graphs and figures with legends) In detail:

7.1 Per cent Seed Germination *in vitro*(%)

When the seeds were soaked in water (a) and treated with *Fusarium* (b) 24 hours, before sown in soil, it was observed that wheat seeds treated with *Fusarium* were subjected to deterioration due to the formation of mycotoxins which caused the change in the quality of seeds and also reduced their germination ability.When the seeds were soaked in water, germination percentage was approx. 96.3%, whereas in *Fusarium* treated seed germination percentage was 82.9% (Fig. 1 A and B, Fig. 2).

7.2 Root and shoot length *in vitro*

In seedlings, soaked in water root length and shoot length was approx. 5.58 cm and 3.01cm whereas in seedlings treated with *Fusarium* root length and shoot length was 3.77 cm and 1.38cm.(Fig. 3 A and B, Fig. 4).

7.3 Screening of Antifungal Activity:

It was observed that when *Fusarium graminearum* were treated with 0.1 µl/ml of clove, neem and peppermint oil, growth inhibition percentage was 24.5%,

13.4% and 7.8% respectively. The growth of colony in clove, neem and peppermint was 6.8 cm, 7.8 cm and 8.3 cm respectively.

When the *Fusarium graminearum* treated with 0.2µl/ml clove, neem and peppermint, growth inhibition was 47.8%, 32.3% and 3.4% respectively. The growth of colony was 4.7cm, 6.1 cm and 6.9 cm respectively (Fig. 5, 10, Table 1). At the concentration 0.5µl/ml, growth inhibition was 88.9%, 64.5% and 50% respectively. The growth of colony was 1cm, 3.2cm and 4.5cm respectively (Fig. 6, 10,Table 1). At the concentration of 1µl/ml, no growth of *Fusarium* was observed in the petri plate treated with clove oil,whereas in other petri plates treated with neem and peppermint growth inhibition was 86.7 % and 56.7% respectively. The growth of colony was 1.2cm and 3.9cm respectively (Fig. 7, 10, Table 1). So we can conclude that 1µl/ml is minimum inhibitory concentration(plate with lowest concentration of oil, showing no visible growth was regarded as MIC) of the clove oil. Similarly 2µl/ml, is minimum inhibitory concentration of the neem oil as no growth of *Fusarium* was observed at this concentration (Fig. 8, 10, Table 1). In peppermint treated petri plate growth inhibition was 75.6% and diameter of colony was 2.2 cm (Fig. 9, 10, Table 1)

Result indicated that different herbal oils have different efficacies. Different oilsposses various components which may be active against different fungal pathogens.Clove oil showed the minimum inhibitory concentration at 1µl/ml, neem at 2µl/ml and peppermint at 5µl/ml. Among all herbal oils clove oil is one of the best oil which can be used as an alternative against the toxic chemicals to prevent the disease. So, clove oil can be used as a natural biocontrol.

Principal component in clove oil is eugenol (80-95%), and therefore its strong antifungal activity may be attributed to eugenol. In neem the active constituent is, whereas in peppermint it is which is active against the funagla pathogen.

Different concentrations of yeast were also applied to observe the growth of *Fusarium*. At 0.1% percent growth inhibition was 45.5% and diameter of colony was 3.9 cm, At 0.2% growth inhibition was 52.2% and diameter of colony was4.3 cm.At 0.3% growth inhibition was 72.2% and diameter of colony was 2.5cm. At 0.4% growth inhibition was 86.6% and diameter of colony was 1.2cm. At 0.5% the complete inhibition was observed (Fig. 11, Table 2).

7.3 Preparation of Field Beds

Field beds were prepared in the back lawns of Shivaji College, Raja Garden. Six field beds were prepared for this experiment. I field bed was for control, II was treated with *Fusarium*, III was treated with *Fusarium* and clove, IV was treated with *Fusarium* and neem, V was treated with *Fusarium* and peppermint and VI was treated with *Fusarium* and yeast treated. Seed were soaked in water 24 hours before sowing in soil. After the emergence of seedlings, foliar spray of herbal oils and yeast were done. Earlier field beds were covered only by cloth net but as the crop were destroyed by monkeys and squirrels it were then completely protected by wired net (Fig. 12).

7.4 Estimation of Chlorophyll

Presence of chlorophyll is an indication of photosynthetic activity. More the chlorophyll more will be the photosynthetic activity. The chlorophyll content was higher in the control seeds (1.56 mg/g) and lowest in the seeds treated with *Fusarium* (1.22mg/g).Among the different treatments, highest chlorophyll content was observed in the yeast treated plants (1.53mg/g) whereas lowest concentration is found in peppermint treated (1.5mg/g). Among the different treatments no significant variation was observed (Figure 13).

7.5 Estimation of Protein

Protein content extracted from seeds was statistically analysed as it is the second most important constituent of wheat. The protein content was higher in the control seeds (14.97%) and lowest in the seeds treated with *Fusarium* (10.16%).Among the different treatments, highest protein content was observed in the yeast treated plants (14.87%) whereas lowest concentration is found in peppermint treated (13.23%). Among the different treatments no significant variation was observed except in peppermint treated (Figure 14).

7.6 Estimation of Carbohydrate

Carbohydrate is one of the major constituents of wheat. The carbohydrate content was higher in the control seeds (57.97%) and lowest in the seeds treated with *Fusarium* (51.19%). Among the different treatments, highest chlorophyll content was observed in the yeast treated plants (57.56%) whereas lowest concentration is found in peppermint treated (55.83%). Among the different treatments no significant variation was observed (Figure 15).

7.7 **Length of Kernel and No. of grains per kernel**

It was observed in control, length of kernel was 12.11 cm and no. of grains per kernel was approx. 15.3. In *Fusarium* treated plants, the length of kernel was 7.11cm and no. of grains per kernel were 9.7 (Fig. 16, 17 and 18)

Discussion

The control of fungal pathogens by chemical fungicides has been of significant help in increasing the crop yield. However, usage of these toxic chemical productsis being discouraged as it leaves toxic residues in soil, water and food. Some chemicals are also harmful to non-target organisms and it leads to ecological imbalance and development of fungicidal resistant strains. All these limitations require an alternative plant disease management strategy i.e. biological control (Armando et al., 2013). Biological control method is preferred because it is selective with no side effects, and is relatively cheap. Moreover, resistance to biological control is rare and biological control agents are self-propagating and self-perpetuating.

Clove (*Syzygium aromaticum* L. Merrill and Perry) is one of the most valuable spices, which belongs to the family Myrtaceae. The dried flower buds have been used traditionally as food preservative and for many medicinal properties like antimicrobial, antifungal and general stimulating, carminative and anesthetic. Eugenol extracted from powdered cloves inhibited the growth of *Aspergillus flavus, A. fumigates, A. acculeatus* and *A. versicolor* (Hitokoto et al., 1980). Clove oil rich in eugenol (approx. 90%) was also reported to inhibit the growth of *Aspergillus niger* (Chaiebet et al., 2007, Pawar and Thaker, 2006). Clove oil exhibits the complete inhibition of mycelial growth of *Botrytis cinerea* also, which cause a great loss to our wine industry (Sirirat et al., 2009; Sessou et al., 2012). It has also been observed that if clove oil and cinnamon oil are mixed in a proper ratio, grapes can be protected against the postharvest decaying fungi such as *Aspergillus niger, Alternaria alternata, Colletotrichum gloeosporioides, Lasiodiplodia theobromae, Phomopsis viticola* and *Rhizopus stolonifer* (Martini et al., 1996). Similarly, the combination of clove oil and cinnamon oils at 3.0% was capable of providing complete protection in rubber wood particle boards against growth of *Aspergillus* sp. and *Trichothecium* sp. for 9 weeks at 25 °C and

100% RH (Yingprasert et al., 2015). In a recent study it was reported that anthracnose caused by *Colletotrichum gloeosporioides* could also be controlled by clove oil (Hong et al., 2015).

Yeast is considered to be a useful biocontrol agent for various fungi like *Aspergillus carbonarius* and *Fusarium graminearum* (Armando et al., 2012) and *Fusarium oxysporum* (Shalaby et al., 2008). The results of our experimental studies depicted that 0.5% yeast had complete inhibition on *Fusarium graminareum.* Plant growth parameters like chlorophyll content, protein and carbohydrate % was found to be in increased quantity after being treated with yeast as compared to the infected plant. Similar results were obtained by Shalaby et al. in 2008 when they found that plant growth parameters increased by the application of *Saccharomyces cerevisiae* as a biocontrol agent of *Fusarium oxysporum* in sugar beet plants.

8. Innovations shown by the project:

The project has been successful in showing comparative results of effect of herbal oils and yeast on the growth of *Fusarium graminearum*. Herbal oils has been considered to be very effective on many fungal disease but no work has yet been done to see the results of effects of different concentrations of clove oil, neem oil, peppermint oil alongwith different concentrations of yeast. Comparative study is very important as there is a dire need of environmental friendly fungicide in not only in our country but worldwide. The results of our study could prove that clove oil is a better option than neem and peppermint. But to make the result more successful among poor farmers, a particular concentration of yeast shows similar effectiveness as any of these herbal oil for reduction of *Fusarium graminearum.*

9. Conclusion and Future direction:

The success of clove oil as fungicide is enormous as compared to any other herbal oil used in this research work. As there is a need for researchers to find cost effective fungicides that can be healthy to environment, this work has huge future benefits to farmers as well as environmentalists. Considering the fact that clove oil is very costly and probably not that cost effective for a poor farmer, it will become a hindrance for farmers. Hence, yeast, which has commendable antifungal activity, is cheap, readily available in market and easily applicable in

fields, is a better alternative as a biocontrol agent. The result of our work is very important considering the health and environment both of which are getting spoiled due to the excessive usage of these toxic chemicals.

In future, there can be direct use of diluted versions of yeast for spraying in large fields to control wheat head blight by *Fusarium graminearum*. If the farmers can afford they may also use herbal oils as biocontrol agents. This will not only save the human health but also will conserve the environment from chemicals.

10. References in APA format:

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11. Publication/s from the work (attach copies):

Talwar S, Mondal N, Mathur P and Bhardwaj S 2016. "Clove Oil: as Biocontrol Agent" In: Proceedings of II International Conference on "Public Health: Issues, challenges, opportunities, prevention, awareness (Public Health 2016)" organized by Krishi Sanskriti, Jawahar Lal Nehru University, Delhi on 21st May (ISBN: 978-93-85822-17-9)

12. Conference Presentation/s (attach copies):

Attended II International Conference on "Public Health: Issues, challenges, opportunities, prevention, awareness (Public Health 2016)" organized by Krishi Sanskriti, Jawahar Lal Nehru University, Delhi on 21st May (Enclosure I) (Oral Presentation).

Attended the Innovation Conclave – 2016 (under the aegis of ANDC Rajatotsav celebrations), organized by Acharya Narendra Dev College, University of Delhi, Delhi on 25th, 26 th October 2016 (Enclosure II) (Poster Presentation).

13. Patent/s and Technology Transfer (attach copies): N.A.

14. Media Coverage (attach copies): N.A.

15. Pictures related to the project (Enclosed)

16. Annexure/Any other information

CLOVE OIL: AS BIOCONTROL AGENT

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Abstract—*India is an agricultural country and ranks second worldwide in farm output. However the economic contribution of agriculture to India's GDP is steadily declining due to loss of standing crop. The major reason for this loss is due to crop diseases caused by fungi, bacteria, viruses and other microorganisms. Limited access to disease-control methods results in annual losses of 30 to 50 percent of the major food crops. The farmers usually rely on chemical insecticides and pesticides for protection of crops from plant pathogens. The excessive usage of these chemicals has resulted in polluting our environment, creating an imbalance in the ecological systems. An alternative method of plant disease management is application of biological controls using herbal compounds extracted from medicinal plants. These compounds are seen to be effective in controlling various plant diseases. Moreover they are environment friendly and have no side effects. Essential oils and their extracts from medicinal plants are able to control various microorganisms causing the diseases in cereal, fruits and vegetables. Essential oils are aromatic, volatile, oily, hydrophobic liquid concentrates that are extracted from plant material, such as flowers, buds, seeds, leaves, twigs, bark, wood, fruits, roots and whole plant. These essential oils contain a variety of volatile molecules such as terpenes, terpenoids and phenol derived aromatic and aliphatic compounds, which have antibacterial, antiviral, and antifungal properties. These essential oils can be therefore used for successful and environment-friendly management of crop diseases, thus increasing the crop yield.*

Clove (Syzygium aromaticum L. Merrill and Perry) is one of the most important spices that have been used from centuries for many medicinal purposes. Active ingredient of clove oil is eugenol, which inhibits the growth of most of the fungal as well as bacterial pathogens. Clove oil has been proved successfully effective in suppressing the growth of Fusarium oxysporum, F. verticillioides, F. avenaceum, F. graminearum, F. moniliforme, Penicillium italicum, P. expansum, P. citrinum, P. viridicatum, Monilinia fructigena, Aspergillus ochraceus, Tricophytonrubrum, and T. mentagrophytes, and Botrytis cinerea as well as many gram positive and gram negative bacteria. Therefore, we can conclude that Clove oil can be used as a source of natural eco-friendly phyto-fungicidal and antibacterial compound.

1. INTRODUCTION

Medicinal plants produce high amount of active principles, which have been used in pharmaceutical industry for their antimicrobial property [1]. These active principles are basically secondary metabolites which are the by-products of primary metabolic pathways. These are viewed as potential source of natural drugs, antibiotics, insecticides and herbicides [2-3]. The medicinal property of most of the plants is associated with the production of essential oil. Essential oils and their extracts have the ability to control various microorganisms (bacteria, fungus, yeast) which cause infection to plants, humans and also spoil the food [4]. These are generally aromatic organic compounds and broadly include phenolics, terpenes, steroids and alkaloids [5]. The active antimicrobial compounds of essential oils are generally terpenes, which are phenolic in nature. These phenolic compounds are responsible for the inhibition of various fungal pathogens [6].

Fungus like *Fusarium* and *Alternaria* have been recognized to be the major plant pathogens all over the world [7]. These fungal plant pathogens results into a great loss to our agriculture every year. Essential oils and their extracts have less toxic side effects than chemical insecticides and pesticides [8]. The pharmacological value of the essential oils is increasing day by day due to their potential role as biocontrol agents, non-phytotoxic compounds and potentially effective against several microorganisms [9-10].

Several experiments have been performed to study the effect of these essential oils on pathogenic fungi. Kurita et al. [11] studied the effect of 40 plant metabolites against seven species of fungal pathogens. Similarly Nosrati et al., [12] have also investigated the antifungal property of spearmint essential oil against *Fusarium oxysporum* f. sp. *radicis-cucumerinum*. Antifungal activity of six essential oils against the *F. oxysporum* f.sp. *radicis-lycopersici* and *F. oxysporum* f.sp. *lycopersici* have also been observed by Arici et al., [13].

2. CLOVE AS ANTIFUNGAL AGENT

Clove (*Syzygium aromaticum* L. Merrill and Perry) is one of the most valuable spices, which belongs to the family Myrtaceae. The dried flower buds have been used traditionally as food preservative and for many medicinal properties like antimicrobial, antifungal and general stimulating, carminative and anesthetic [14-17]. Clove plant is the native of Indonesia but nowadays it has been cultivated in many parts of the world [18].

Eugenol (80-95%), acetyl eugenol (1-5%), and β-caryophyllene (4-12%) are main chemical components of

ISBN-978-93-85822-17-9

Innovation Conclave: 25 – 26 October 2016
Acharya Narendra Dev College (University of Delhi), Kalkaji, New Delhi – 110 019

Poster Presentation – LS-5

Antifungal activity of some essential oils against *Fusarium Graminearum*

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Abstract

India is an agricultural country and its economy is chiefly dependent on agriculture. In spite of economic development and industrialization, agriculture still forms the backbone of the Indian economy. Nearly two-third of India's population is dependent directly on agriculture for its livelihood. Though over 50% of the area is under cultivation in India, the yield in agriculture is low. Wheat forms the staple food of millions of Indians, predominantly in the northern and north-western parts. A number of diseases caused by microbes (fungi, bacteria and viruses) results in large losses in wheat yield. This reduction in the yield from plant pathogens results in hunger and starvation. To avoid this destruction, growers often rely on chemical insecticides and pesticides. These chemicals, no doubt help in increasing production but their use also results in environmental pollution, various ill effects to biotic community, and many hazards to the ecological systems. Therefore, the biological method of plant disease management and control seems to be a better alternative to these toxic chemicals. Awareness of the harmful effects of these chemicals to human health and environment has led to application and use of natural plant extracts becoming increasingly important to control plant diseases. Essential oils and extracts obtained from many plants have recently gained great popularity and scientific interest. So plant essential oils form potential sources of antimicrobials of natural origin. The present study was conducted to find out the antifungal activity of essential oils of Clove, Neem and Peppermint against the fungi *Fusarium graminearum* which causes head blight disease of wheat. Among the clove, neem and peppermint oils, clove oil was found to be the best as it showed the highest antifungal activity at 1µLml-1. At this concentration clove essential oil caused complete inhibition of growth of *F. graminearum*.

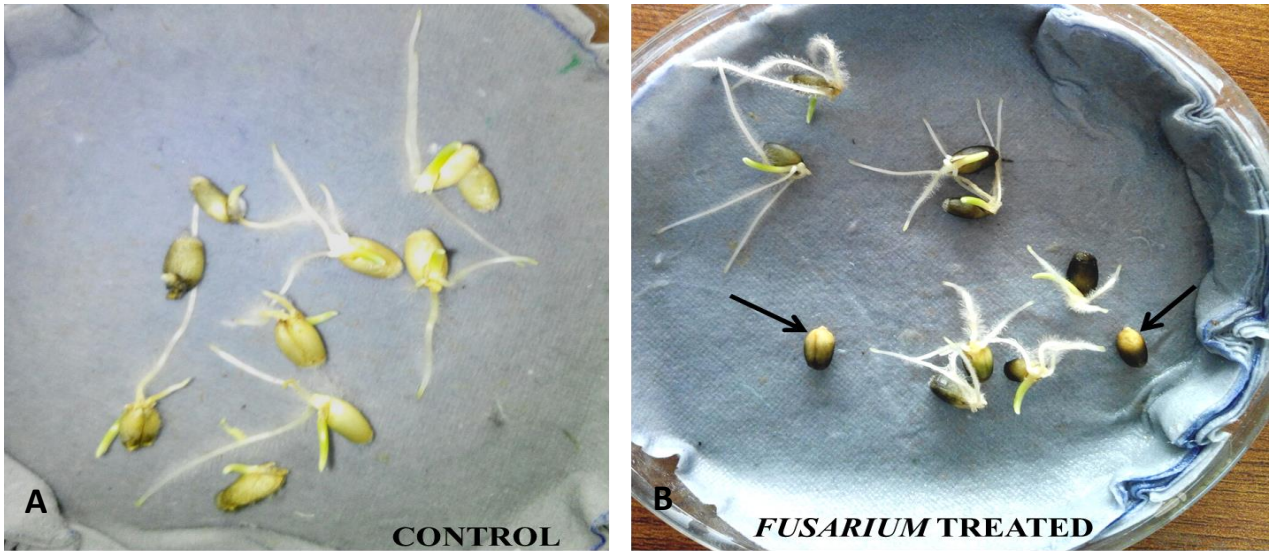
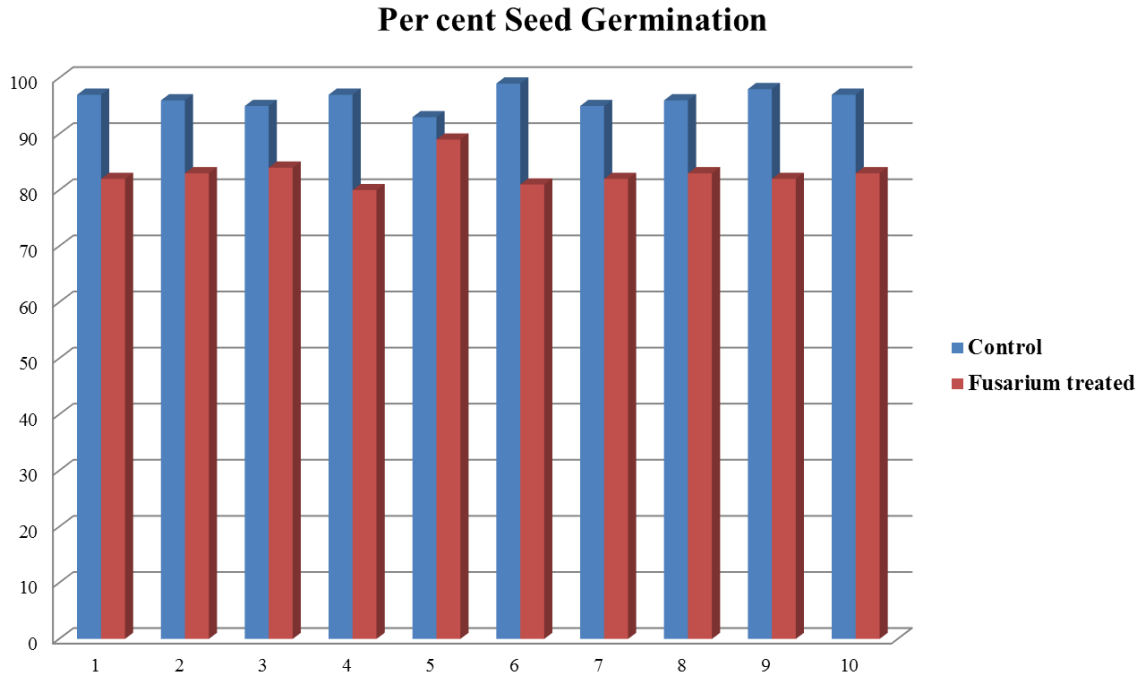


FIG. 1 A: Petri plate showing seed germination in control.

Fig. 1 B: Petri plate showing seed germination after treatment with *Fusarium*. Arrow indicating no seed germination, most of the seeds are shriveled and black in colour.



Average Control seed germination (%) : 96.3±1.70
Average *Fusarium* treated (%) : 82.9±2.42

Fig. 2: Graph showing the per cent seed germination in control and *Fusarium* treated seeds.

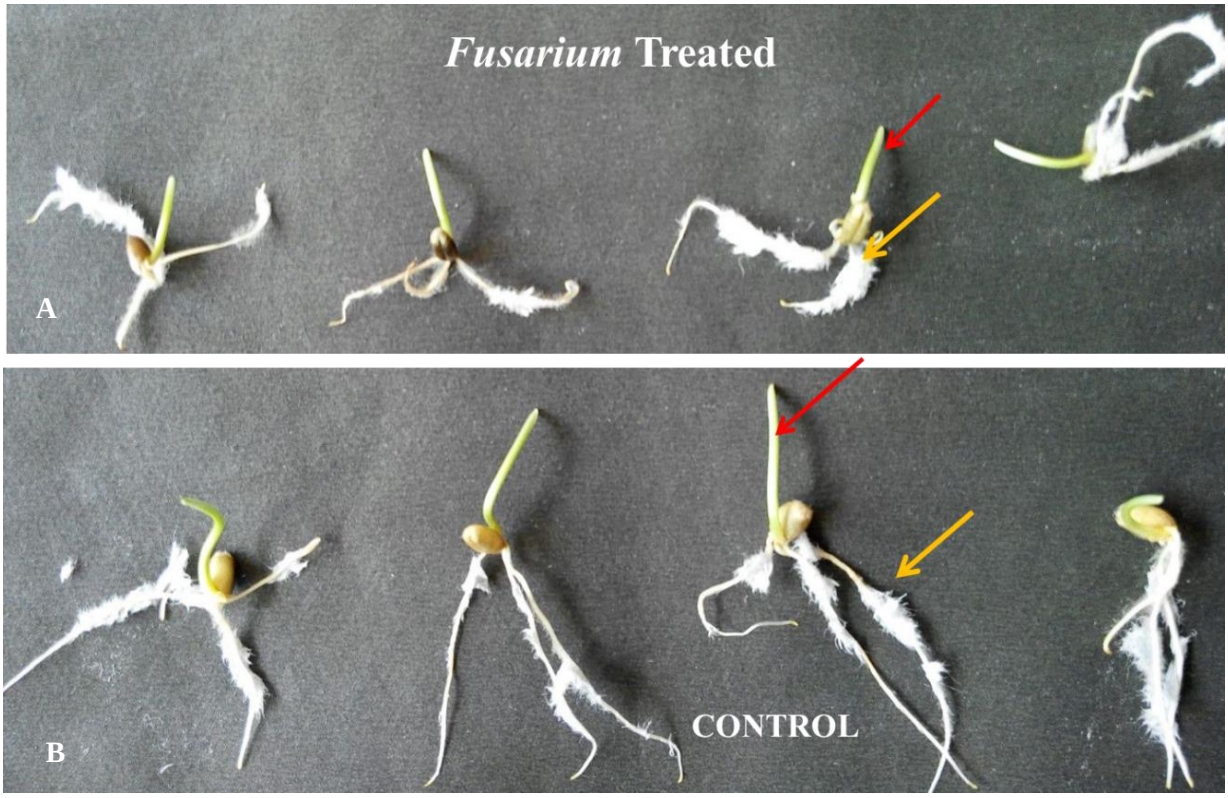


Fig. 3A: Picture depicting the length of root and shoot *in vitro* in *Fusarium* treated seed (Red arrow indicating the length of plumule, yellow indicating the length of radicle)

Fig. 3B: Picture depicting the length of root and shoot *in vitro* in control seed (Red arrow indicating the length of plumule, yellow indicating the length of radicle)

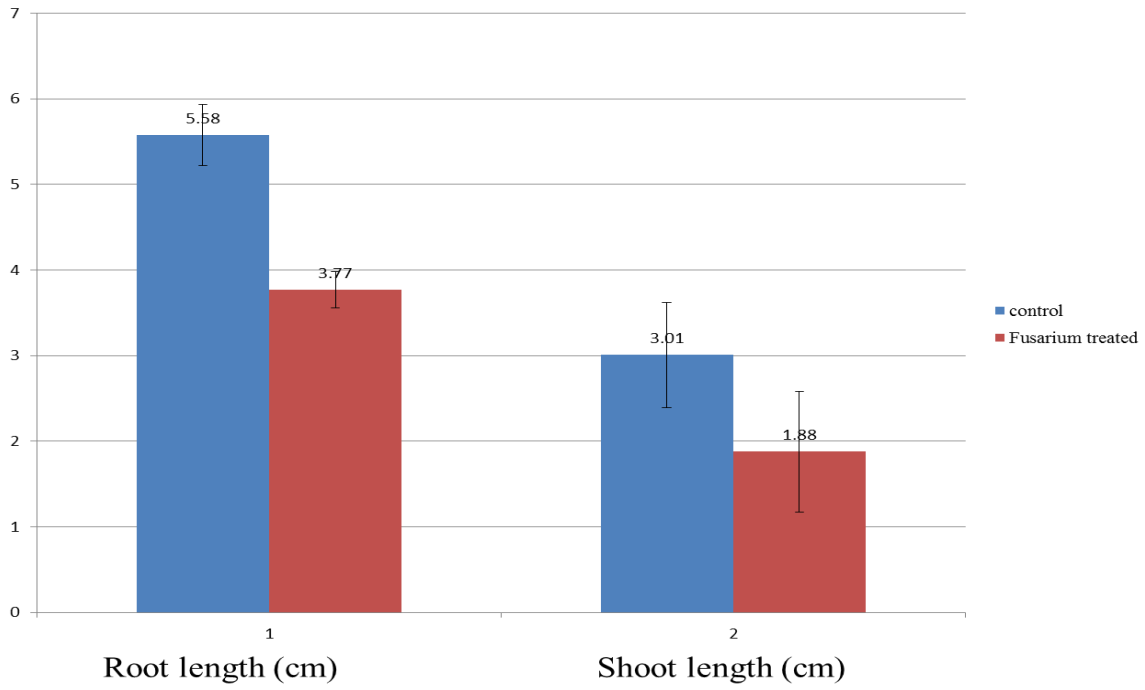


Fig. 4: Graph showing length of root and shoot *in vitro* after 72 hours.

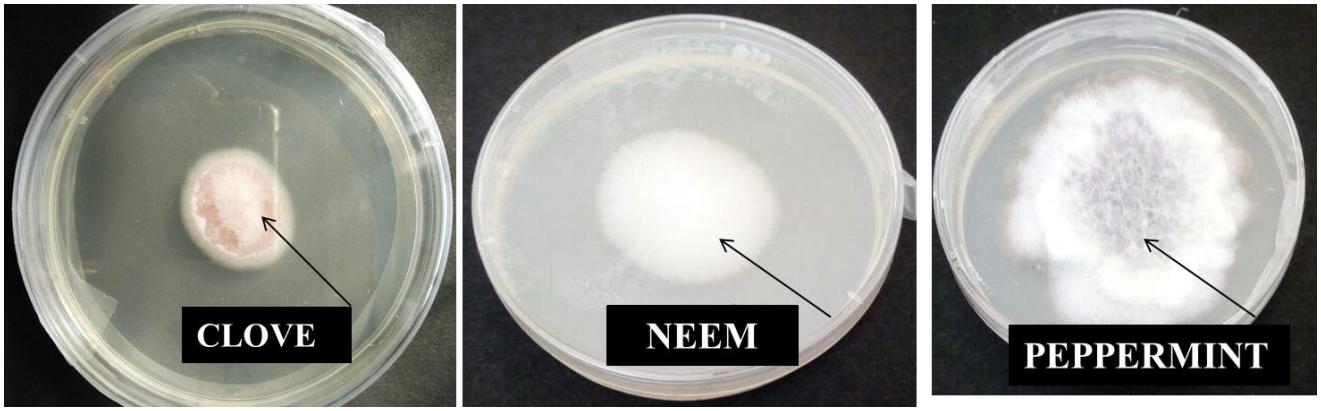


Fig. 5: Petri plates showing the growth of *Fusarium graminearum* at the concentration of 0.2 µl/ml of clove, neem and peppermint oil.

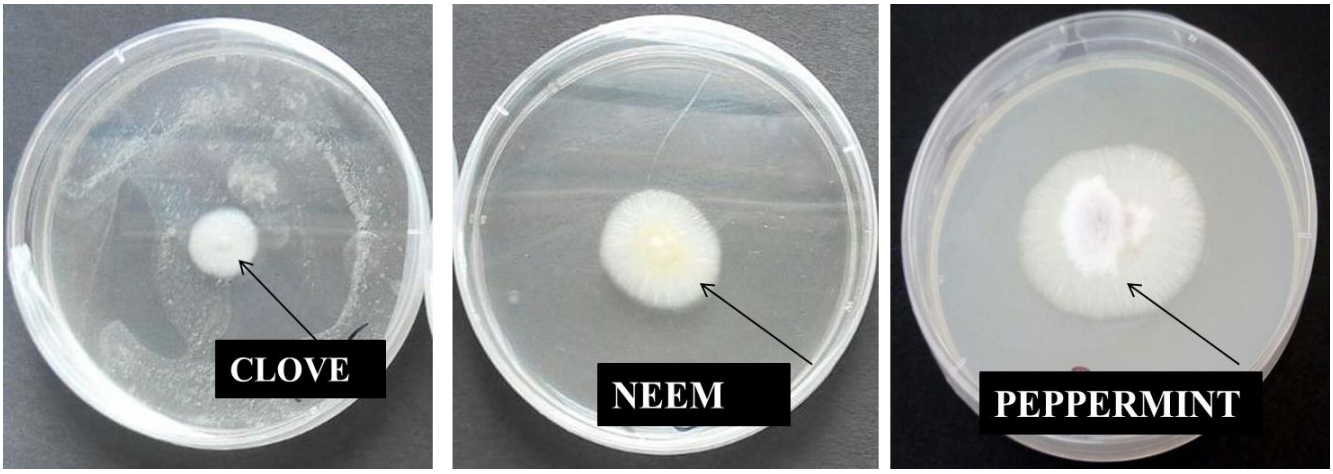


Fig. 6: Petri plates showing the growth of *Fusarium graminearum* at the concentration of 0.5 µl/ml of clove, neem and peppermint oil

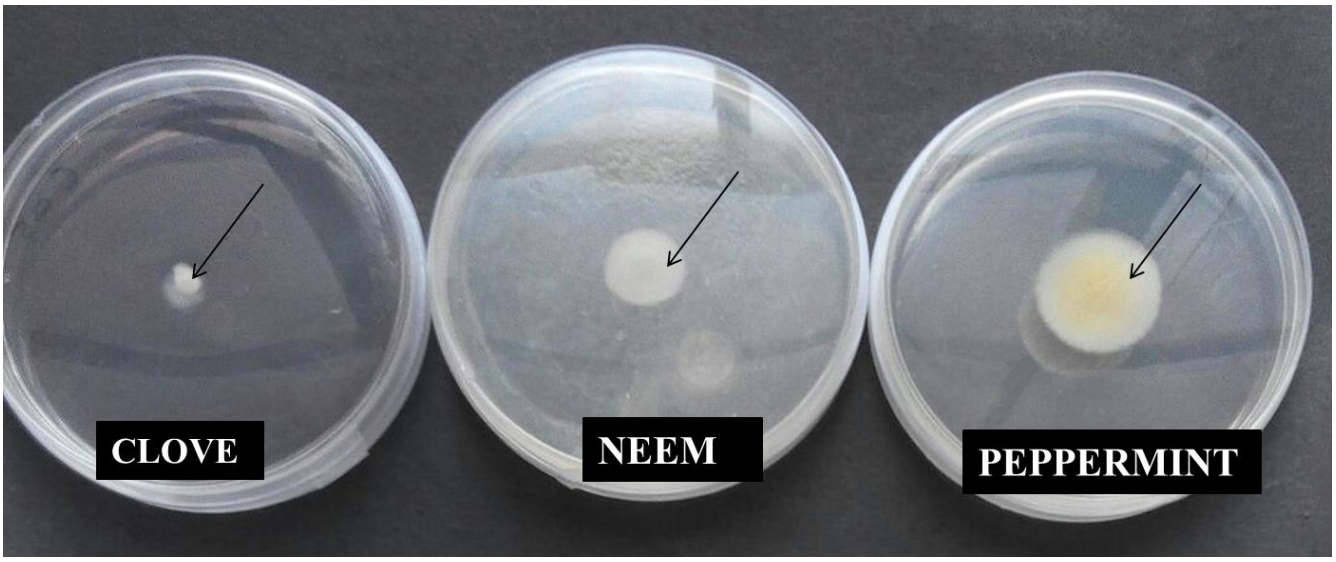


Fig. 7: Petri plates showing the growth of *Fusarium graminearum* at the concentration of 1µl/ml of clove, neem and peppermint oil

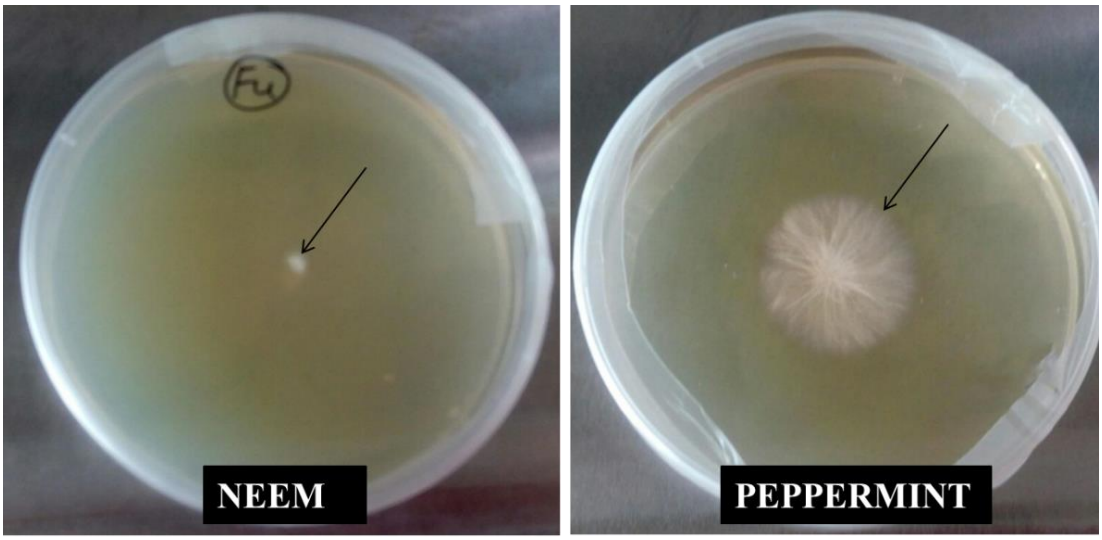


Fig. 8: Petri plates showing the growth of *Fusarium graminearum* at the concentration of 2 µl/ml of neem and peppermint oil.

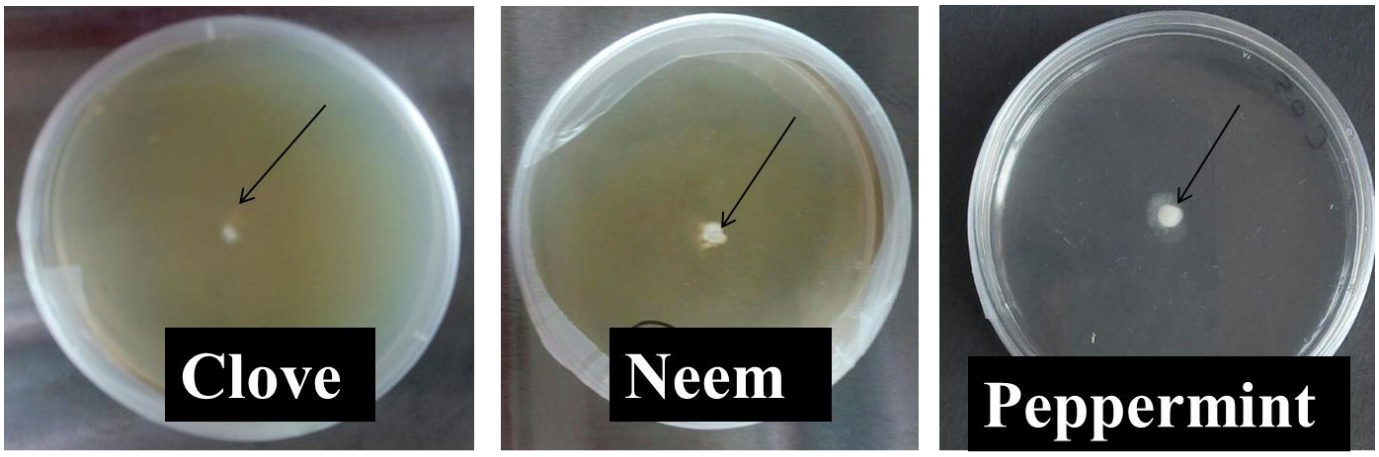


Fig. 9: Petri plates showing the complete inhibition of *Fusarium graminearum* at the concentration of 5 µl/ml of clove, neem and peppermint oil.

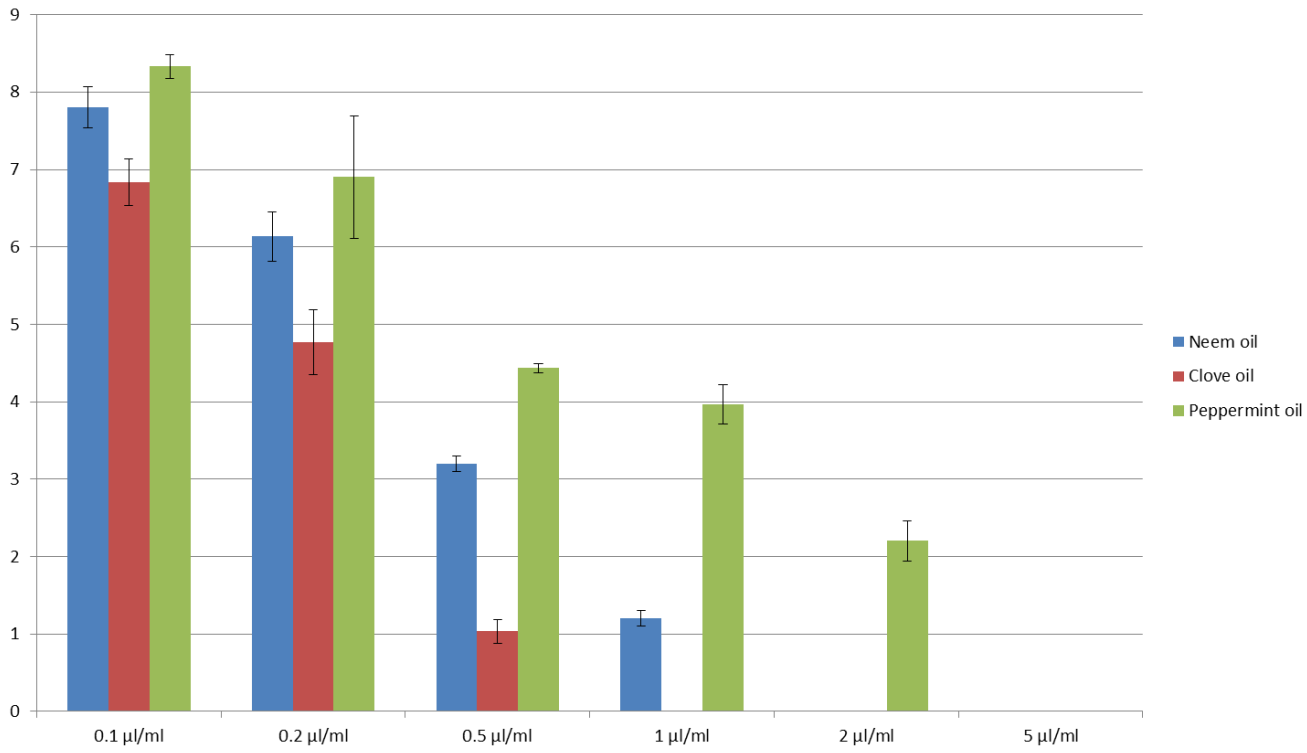


Fig. 10: Graph showing the antifungal activity of clove, neem and peppermint oil against *Fusarium gramineaum*

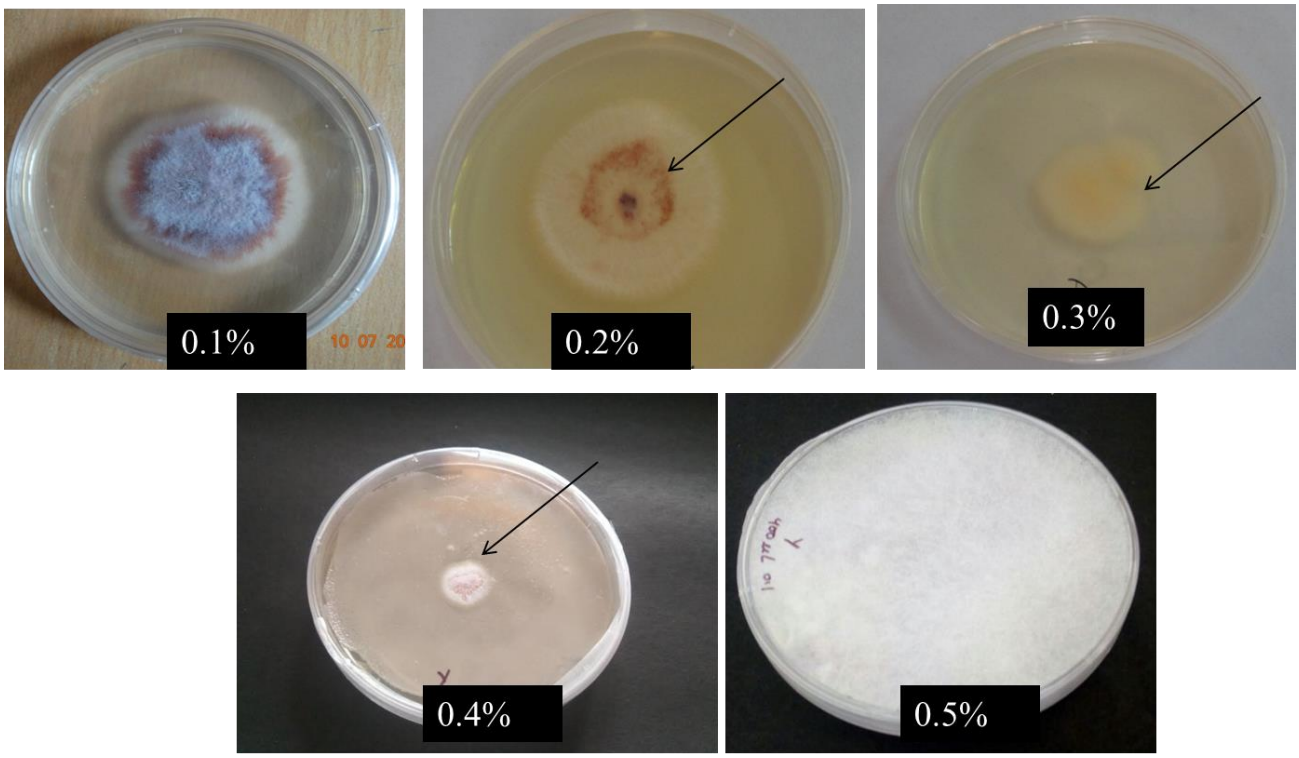
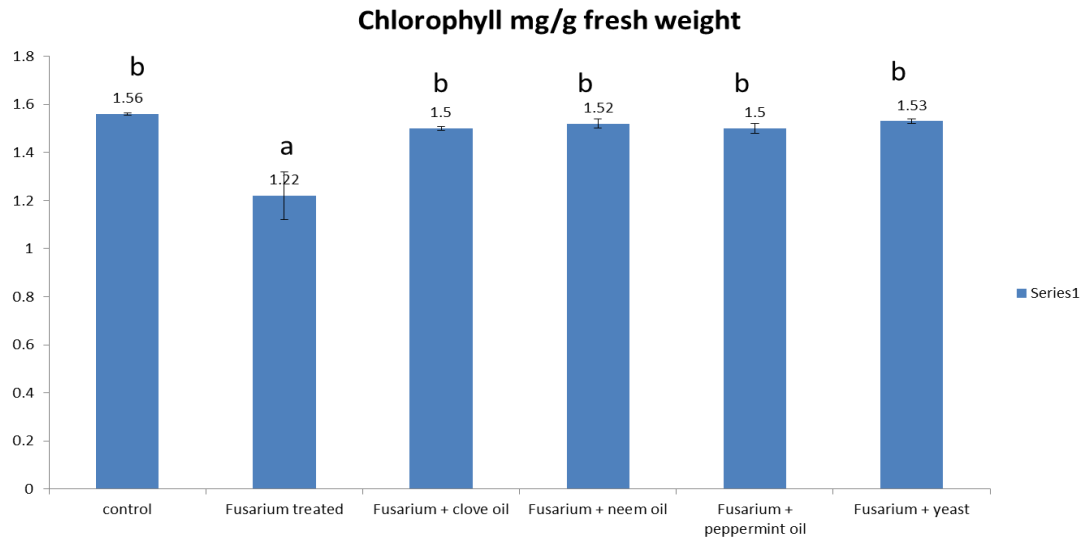


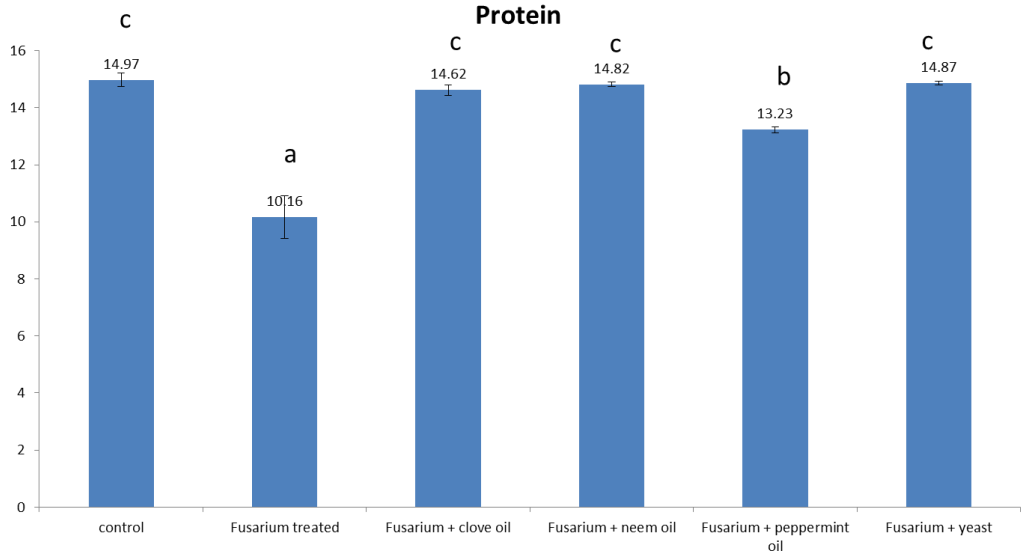
Fig. 11: Petri plates showing the growth of *Fusarium graminearum* at different concentrations of yeast i.e. 0.1, 0.2, 0.3, 0.4 and 0.5%.

Fig. 12: Photographs showing the field beds at back lawns of Shivaji College, University of Delhi (covered by net and wire).



There is significant difference between chlorophyll fresh weight ($F_{(5,12)} = 33.277, P = 0.001$; One Way ANOVA). In figure a,b,c are different subsets showing comparison among subsets using post-hoc Tukey's test. Fusarium treated is significantly different from all others. Rest all show no difference among themselves.

Fig. 13: Graph showing the estimation of chlorophyll.



There is significant difference between the Proteins ($F_{(5,12)} = 94.52, P = 0.001$; One Way ANOVA). In figure a,b,c are different subsets showing comparison among subsets using post-hoc Tukey's test.

Fig. 14: Graph showing the estimation of protein.

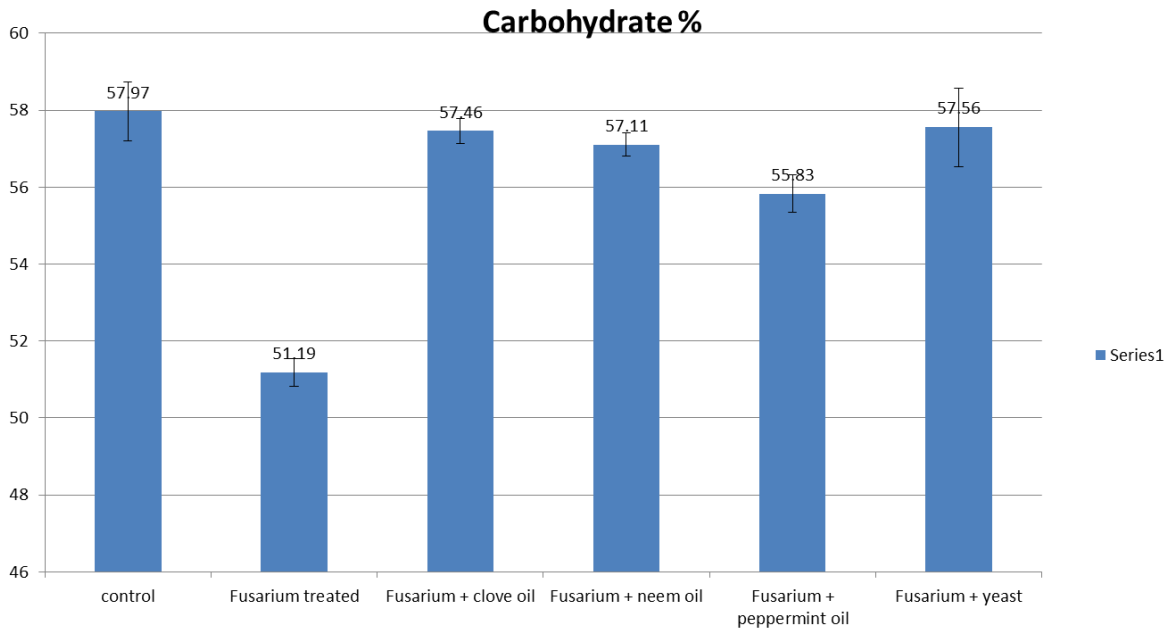
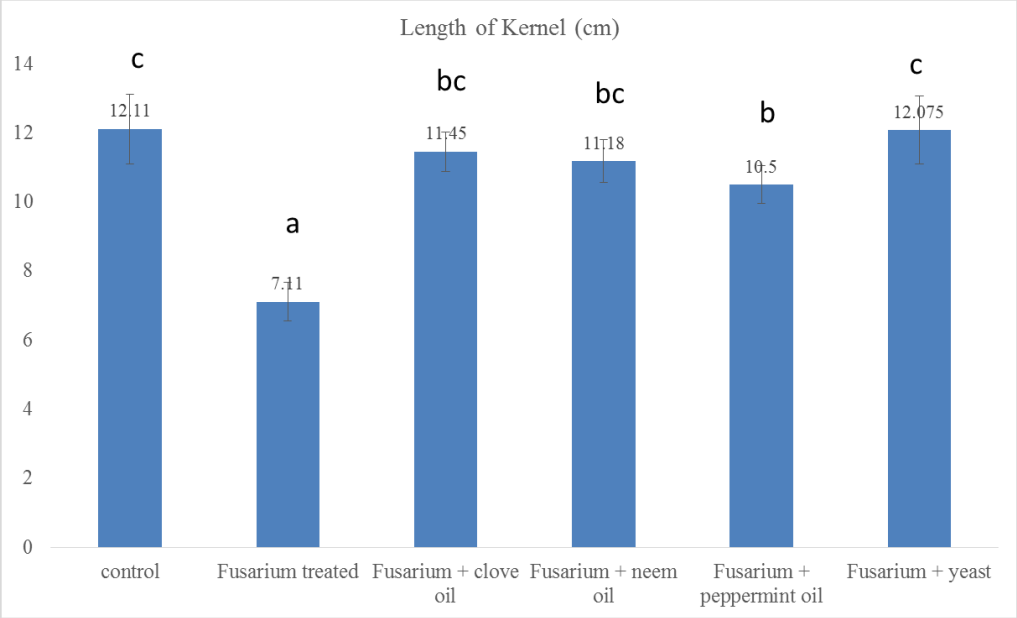


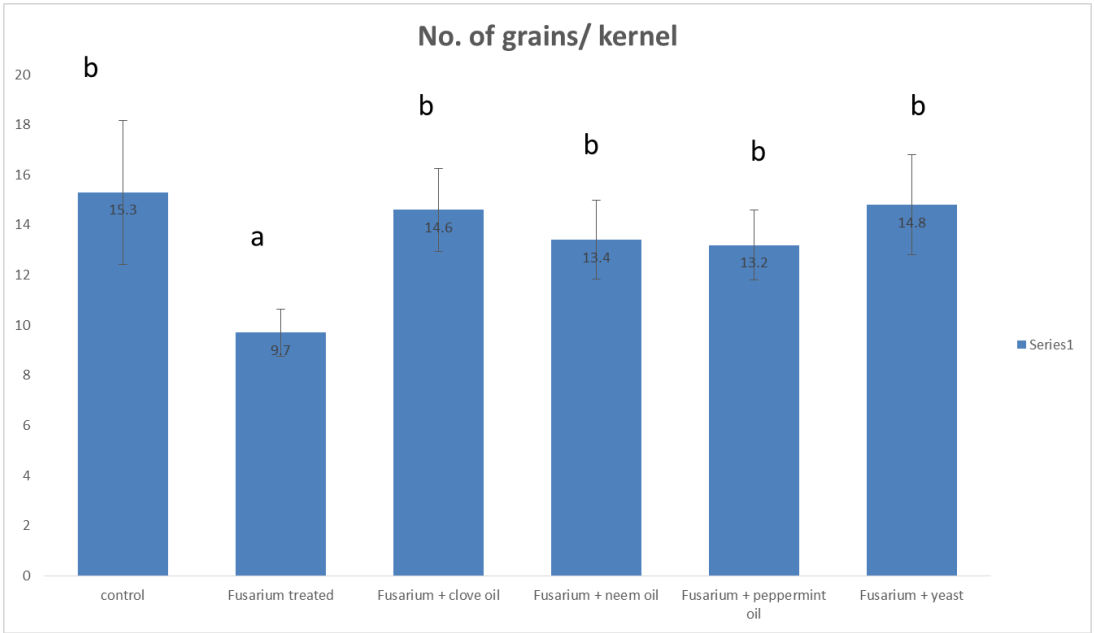
Fig. 15: Graph showing the estimation of carbohydrate.

Fig. 16: Picture showing the difference in the length of kernel.



There is significant difference between the length of kernels ($F_{(5,54)}$ = 63.678, P = 0.001; One Way ANOVA). In figure a,b,c are different subsets showing comparison among subsets using post-hoc Tukey's test.

Fig. 17: Graph showing the difference in the length of kernel.



There is significant difference between the No.o grains per kernels ($F_{(5,12)}$ = 12.26, P = 0.001; One Way ANOVA). In figure a,b,c are different subsets showing comparison among subsets using post-hoc Tukey's test.

Fig. 18: Graph showing the number of grains/ kernel.




Photographs of students working in field and laboratory.


Photographs of meeting with mentor of project.



Wheat plant infected with *Fusarium graminearum*, arrow indicating the initiation of disease