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Role of biocontrol agents on chlorophyll content in wilt affected lentil crop

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Abstract
In the present study biocontrol agents *Bacillus safensis* and *Bacillus licheniformis* has been isolated from wilt affected lentil crop of agro-climatic zone of eastern UP like Faizabad, Mau, Varanasi, Gazipur.Lentil seed treatment has been given by these isolated strains as bioagents against wilt pathogen in pot experiment in lentil crop Rabi-season 2019-2020.Result of conducted pot experiment showed the maximum chlorophyll content was found in T3 (*Bacillus safensis* +*Bacillus licheniformis* +*Fusarium oxyporum f.sp.lentis*+Lentil Rasistant variety IPL-316 + Susceptible variety K-75.) followed by treatments T2 (*Bacillus licheniformis* +Resistant Variety IPL-316+Susceptible variety K-75) and T1 (*Bacillus safensis* +Lentil Resistant variety IPL-316 + Susceptible Variety K-75) as compare to negative control T5 (Lentil Resistant variety IPL-316 + Susceptible Variety K-75+No bacterium inoculum) and pathogen T4(*Fusarium oxysporum f.spp.lentis* + Resistant variety IPL-316 + Susceptible Variety K-75. The aim of this paper is to study the bio-agent's factor affecting the chlorophyll content and provide protection against pathogen in lentil plant. After that chlorophyll estimation test was performed on treated lentil crop on 30, 60 and 90 days. We observed chlorophyll content in treated crops and maximum chlorophyll content was found at 30 and 60 *days which minimize at 90 days*.

Keywords: *Bacillus safensis*, *Bacillus licheniformis*, *Fusarium oxysporum f.spp. lentis*, Lentil seed resistant variety (IPL-316), Lentil seed susceptible variety (K-75)

Introduction
Lentil (*Lens culinaris Medik*.) is a member of Leguminaceae family and commonly known as legume, masoor or poor man's meat. The global production of lentil has reached nearly 5.0 million tonnes in 2014 (Ahmad Nasim 2018) [4]. It is the second largest growing rabi pulse after chickpea in India. Lentils *(Lens culinaris Medikus*) produces commonly in cold weather which is an important pulse crop grown widely throughout the Indian subcontinent, Canada, Turkey, Australia, USA, Nepal, China and Ethiopia. About 80 percent of the lentil production in India during 2015-16 has been reported from Madhya Pradesh (30.94%), Uttar Pradesh (28.72%), Bihar (15.24%) and West Bengal (5.81%) (Ahmad *et al.*, 2018). Lentil has a high nutritional value and major source of dietary proteins (25%) after soyabeans in humans and animal diet (Rahman *et al*., 2010) [5] and also plays a significant role in the improvement of soil fertility (Frederick *et al.*, 2006) [1].

Fusarium wilt disease caused by pathogen *Fusarium oxysporum f.sp. lentis* is the most important biological constraints to productivity of lentil worldwide (Bhalla *et al.*, 1992) [2] and is considered as to be the most important biotic stress affecting the crop's productivity (Khare, 1981) [3]. It is a soil borne root pathogen, colonizing the xylem vessels and blocking them completely and in this way it prevents water to reach towards the other parts of the crop. Wilt symptoms appear from flowering to late pod-filling stage and are characterized by sudden drooping of top leaflets of the affected plant, leaflet closure without premature shedding, dull green foliage followed by wilting of the whole plant or individual branches. This pathogen can cause infection at all the stages of plant growth with more incidences at flowering and podding stages than early vegetative stage (Chavdarov, 2006) [6]. As the pathogen *F. oxysporum* can survive for longer period in soil and difficult to eliminate, it may cause severe obstructs to the plant's xylem hence deficit the water in leaves and also lowers the gas exchange properties

(stomatal conductance, transpiration, and photosynthesis) which results low plant growth, leaf area and dry matter accumulation, followed by vascular tissue wilting and finally plant death.

Chlorophyll is a pigment responsible for green colour found in plants and algae (Aminot, 2000) [7]. Chlorophyll and its derivatives also have profound antioxidant properties. Chlorophyll derivatives such as pheophorbide and pheophytin b have always been known as strong antioxidants. Indirect evidence is also there that chlorophyll is jointly controlled by climate and soils thus chlorophyll might be an indicative trait for characterizing how plants respond to climate change (Ying Li *et al.,* 2018) [9]. The process of photosynthesis is vital to the production of food and fibre as it provides the raw materials for all plant products. In these compatible interactions, the virulent pathogen can spread in the susceptible plant. Specific resistance is based on the recognition of the activity of these effector molecules by plant receptor proteins. In these incompatible interactions the plant is resistant and can successfully prevent the pathogen spreading. The successful defense is based on the early recognition of avirulent strains of plant pathogens and the fast activation of defense (Jones and Dangl, 2006) [11]. Furthermore, the recognition of the avirulent strains activates, formed and induced defense mechanisms. In addition to the already mentioned defense reactions, a localized programmed cell death which can efficiently halt the spreading of biotrophic pathogens (Heath, 2000) [10]. Recognition of the presence of micro-organisms is the first step for the activation of defense responses.

Due to weaker defense system and efficiency of virulent, disease pathogen attack on root and block the xylem tissue which is very important part of the plants. It is used mostly for transporting water from roots to stems and leaves but also transports other dissolved compound. Due to wilt disease then lack of water in leaves were damage the foliage leaves and also affected the chlorophyll content in lentil crop.

So the biocontrol agents can suppress these pathogens and enhances the efficiency of defense mechanisms in plants. The biocontrol agents produce PR proteins against pathogen and inhibit its activity and showing PGPR activity which promotes growth of plant.

Hence keeping the positive impact of biocontrol agents on wilt diseases of lentil the present study was conducted. Rhizospheric strains from wilt affected lentil crop of agro-climatic zone of eastern UP like Faizabad, Mau, Varanasi, Gazipur has been isolated. Lentil seed treatment has been given by these isolated strains as bio-agents against wilt pathogen in pot experiment in lentil crop Rabi-seaseon 2019-2020. These bio-agents provide protection for lentil plant. In this treatment we found maximum chlorophyll content in T3 resistant variety IPL-316 and susceptible variety K-75 (Bacillus *safensis* and Bacillus *licheniformis*) and follwed by T2 (Bacillus *licheniformis*) in compare to negative control T5 and pathogen T4.The aim of this paper is to study the bio-agent's factor affecting the chlorophyll content and provide protection against pathogen in lentil plant. After that chlorophyll estimation test was performed on treated lentil crop on 30, 60 and 90 days. We observed chlorophyll content in treated crops and maximum chlorophyll content was found at 30 and 60 days which minimize at 90 days.

Materials and methods

Treatments

T1: *Bacillus safensis* bacteria + Lentil Resistant Variety (IPL-316) + Susceptible Variety (K-75)

T2: *Bacillus licheniformis* bacteria+ Lentil Resistant Variety (IPL-316) + Susceptible Variety (K-75)

T3: *Bacillus safensis* + *Bacillus licheniformis* + *Fusarium oxyporum f.sp.lentis*+ Lentil Resistant Variety (IPL-316) + Susceptible Variety (K-75)

T4: *Fusarium oxysporum f.sp.lentis* +Lentil Resistant Variety (IPL-316) + Susceptible Variety (K-75)

T5: Negative control (no bacterial culture) + Lentil Resistant Variety (IPL-316) + Susceptible Variety (K-75)

T6: Positive control (*Bacillus subtilies*) + Lentil Resistant Variety (IPL-316) + Susceptible Variety (K-75)

Seed bacterization

0.1% - mercuric chloride
D/W - 1000ml
Bacillus inoculums - 3×10^8 cfu ml^{-1}
Seeds - 10 -20 /pot
Soil sample - 3 kg/pot

Chlorophyll Extraction material

1. Plant extracts
2. 80% Aqueous acetone
3. Calcium carbonate

Procurement of wilt disease Pathogen (*Fusarium oxysporum f.spp. lentis*)

Wilt disease caused pathogen (*Fusarim oxysporum f.spp. lentis*) procured from the Indian Institute of Pulses Research Kalyanpur Kanpur India (UP).

Procurement of positive bio-agent (*Bacillus subtilis*)

Positive bio-agent *Bacillus subtilis* provide resistant against wilt disease procured from the National Bureau of Agriculturally Important Microorganisms, Mau Nath Bhanjan-275103 (UP) India.

Preparation of microbial inoculums

The pathogen inoculum was prepared by culturing the fungus, *Fusarium oxysporum f.spp.lentis* on PDA medium for 7 days in petri plates. The microconidial suspension was prepared by pouring 20 mL of sterile distilled water in each petri plate. The concentration of microconidia was adjusted to 1000 conidia mL^{-1}.

The bacterial and fungal inoculum was prepared by inoculating them in respective broth followed by incubation at 28 °C for 48 h at 150 rpm. Population was then adjusted to 3×108 colony forming units (cfu) as measured by spectrophotometer.

Procurement of seeds of Lentil

Seeds of wilt disease sensitive lentil (IPL-316) resistant variety were procured from the Department of Plant Breeding and Genetics, Narendra Deva University of Agriculture and Technology, Kumarganj, Ayodhya (UP) and (K-75) susceptible variety were procured from the Indian Institute of Pulses Research, Kalyanpur, Kanpur, (UP) India.

Soil preparation

Medium black clayey soil from local lentil field was collected and autoclaved 3x (1h, 121 °C) at 12 hrs-intervals. 3 kg of sterilized soil was filled per pot. For challenge inoculation with the pathogen, microconidial suspension (1000 conidia mL-1) of *Fusarium oxysporum f.spp.lentis* was mixed with sterilized soil @ 50 ml/kg before filling the pots.

Lentil seed bacterization

Seeds were surface-sterilized with 0.1% mercuric chloride for 30 seconds and washed 5-6 times with sterile distilled water and dried under a stream of sterile air. 10 ml of *Bacillus spp*. inoculum containing 3×10^8 CFU ml^{-1} was added to petri plates. For triple inoculation *Bacillus safensis, Bacillus licheniformis* and pathogen inoculum were mixed in 1:1:1 ratio. Seeds were soaked in 10 mL of bacterial suspension for 3-4 h. Then, the bacterial suspension was drained off and the seeds were dried in sterile petri plates and shown 10 seeds/bag. Seeds imbibed in sterile water without any further treatment served as control.

Extraction of chlorophyll

The chlorophyll a and b as well as total content was estimated following the method of Arnon (1949) [8] and expressed as mg per g fresh weight.

1 gram of leaf sample was mixed with 80% (v/v) acetone and pinch of calcium carbonate and homogenized in a pre-cooled mortar and pestle. The extract was centrifuged at 3000 rpm for 15 minutes and the volume was made up to 2 ml with 80% acetone. OD was measured at 645 nm and 663 nm against 80% acetone as blank.

Calculation

Chlorophyll a (µ/g/ml) = (12.7 x OD at 663 nm) – (2.69 x OD at 645 nm)

Chlorophyll b (µ/g/ml) = (22.9 x OD at 645 nm) – (4.08 x OD at 663 nm)

Total Chlorophyll (µ/g/ml) = (20.2 x OD at 645 nm) + (8.02 x OD at 663 nm)

Statistical analysis: Statistical analysis was done by One-way ANOVA.

Result & Discussion

All the bio-agents treatment in resistance variety (IPL-316) and susceptible variety (K-75) enhanced chlorophyll content at all the stages of observation in comparison to negative control. All the bio-agent treatments significantly increased the chlorophyll content at 30, 60 and 90 DAS over negative control (T5) while minimum values were found in group treated with pathogen only (T4). Significantly higher chlorophyll content was analyzed with bio-agents in resistant variety (IPL-316) *Bacillus safensis and Bacillus licheniformis spp*. (T3) as 5.10, 8.41 and 10.16 mg and in susceptible variety K-75, 4.97, 7.20, and 8.69 mg g-1 were record at 30 DAS, 60 DAS and 90 DAS respectively. The next highest chlorophyll content was estimated in leaves when *Bacillus licheniformis* (T2) was applied to the pot. In resistant variety IPL-316 the chlorophyll content was recorded as 4.80, 6.82 and 8.27 and in susceptible variety 4.41, 6.26 and 7.58 at 30 DAS, 60 DAS and 90 DAS respectively. All the bio-agent treated pots gave significantly higher value in comparison to negative control T5 value (5.08, 7.36 and 8.89 mg g-1) in case of wilt affected susceptible variety K-75 values are 4.50, 6.52 and 7.88. In all bio-agents treatment, minimum chlorophyll content was found in pathogen *Fusarium oxysporum f.sp. lentis* (T4) as in resistant variety (IPL-316) 4.24, 5.93 and 7.20 and in susceptible variety K-75 4.12, 3.82 and 2.83. It is clear from the (Fig: 1 a, b & c, table: 1 and graph no: 1). Similar effects of fertilizers and bio-fertilizers on photosynthetic pigments of paddy was reviewed. According to other researchers, the highest content of chlorophyll a (0.821 mg/g fr. wt.), chlorophyll-b (0.671 mg/g fr. wt.), total chlorophyll (1.598 mg/g fr. wt.) and carotenoid (0.721 mg/g fr. wt.) were recorded in paddy crop grown in soil treated with *Azospirillum* and *Bacillus*. Similarly, the lowest chlorophyll a, chlorophyll b, total chlorophyll and carotenoid content (0.378, 0.262, 0.640 and 0.359 mg/g fr.wt. basis) were recorded in paddy crop grown without fertilizers. Chlorophyll a, b and total chlorophyll content is an indicative of photosynthetic and metabolic activity. The disappearance of chlorophyll is the one of the most prominent phenomenon of an advanced age and rate of chlorophyll degradation. It is considered a reliable criterion of age-related deterioration and loss of essential plant metabolites.

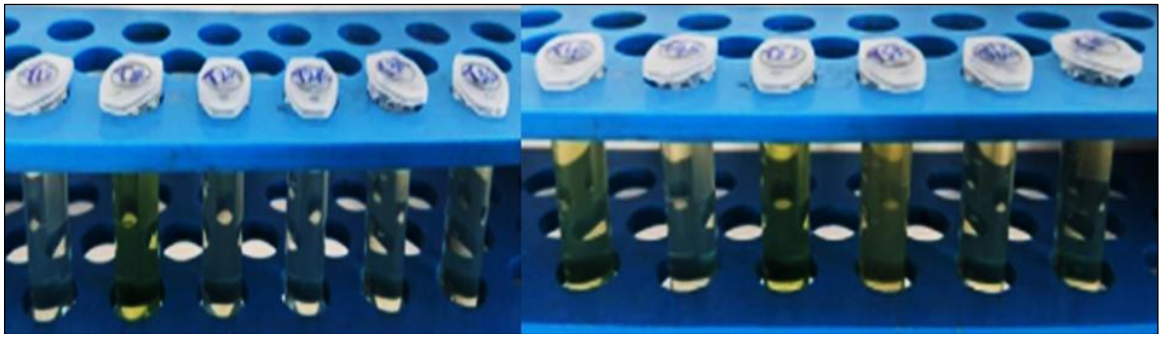


Fig 1 A: Estimation of chlorophyll content in lentil leaves treated with bio control agents and fungicide using DMSO on 30th Days

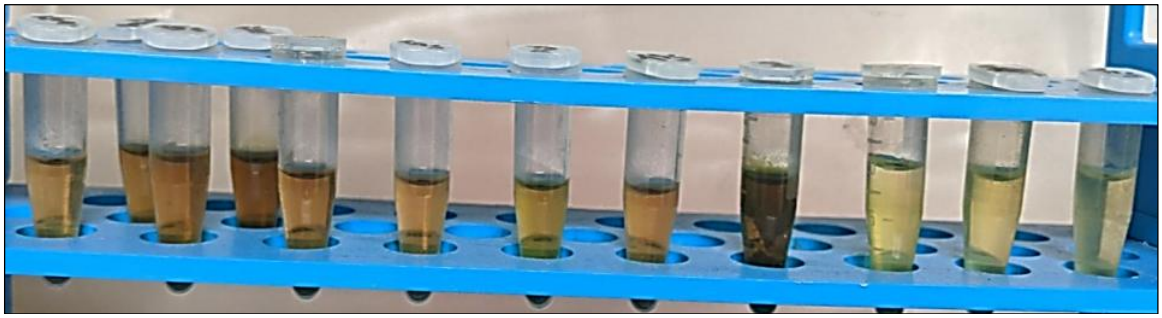


Fig 1 B: Estimation of chlorophyll content in lentil leaves treated with bio control agents and fungicide using DMSO on 60th Days

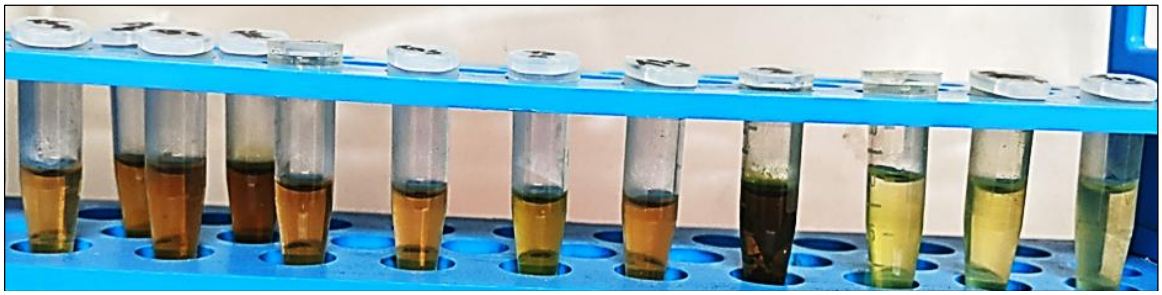
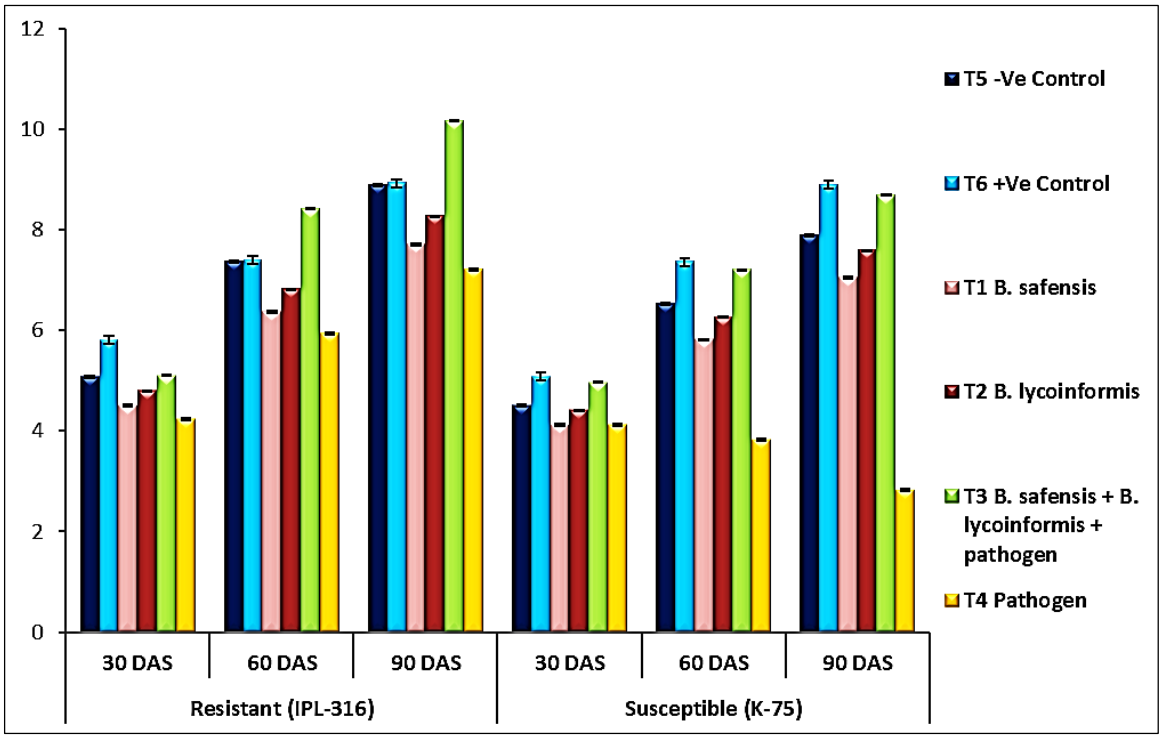


Fig 1 C: Estimation of chlorophyll content in lentil leaves treated with bio control agents and fungicide using DMSO on 90th Days

Table 1: Estimation of total chlorophyll content (µg/ml) seed treated lentil resistant variety IPL-316 and susceptible variety K-75 at 30, 60 and 90 Days

S.No	Treatments	Total chlorophyll content (µg/ml)					
		Resistant (IPL-316)			Susceptible (K-75)		
		30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
T5	-Ve Control	5.08	7.36	8.89	4.50	6.52	7.88
T6	+Ve Control	5.80	7.39	8.92	5.08	7.36	8.89
T1	*B. safensis*	4.51	6.36	7.71	4.12	5.81	7.05
T2	*B. licheniformis*	4.80	6.82	8.27	4.41	6.26	7.58
T3	*B. safensis* + *B. licheniformis* + pathogen	5.10	8.41	10.16	4.97	7.20	8.69
T4	Pathogen	4.24	5.93	7.20	4.12	3.82	2.83
	SEM±	0.02	0.08	0.02	0.01	0.01	0.02
	CD at 5%	0.05	0.18	0.03	0.173	0.12	11.72



Graph 1: Graph showing total chlorophyll content (µg/ml) in treated lentil resistant variety IPL-316 and susceptible variety K-75 at 30, 60 and 90 Days. All the data were represent the mean ± SEM

*Significant differences were found at $p<0.05$

Reference

1. Freidrick *et al*. Application of biotechnology in breeding lentil for resistance to biotic and abiotic stress Euphytica. 2006; 147:149-165.
2. Bhalla MK, Nozzolillo C, Schneider E. Observation on the responses of lentil root cells to hypha of Fusarium oxysporum. J Phytopathol. 1992; 135:35-341.
3. Khare MN, Farnham Royal UK. Commonwealth Agricultural Bureaux. Aleppo, Syria: ICARDA. In: *Lentils*, 1981, 163-172 (eds. C. Webb and G. Hawtin.
4. Ahmad Nasim *et al.* Economic Analysis of Production and Instability of Lentil in Major Lentil Growing States of India ISSN: 2320 – 7051 Int. J Pure App. Biosci. 2018; 6(1):593-598.
5. Rahman *et al.*, Physiological Study and both *In vitro* and *In vivo* antifungal activities against Stemphylium botryosum causing Stemphylium blight disease in lentil (*Lens culinaris*). Plant Pathol. J. 2010; 9:179-187.
6. Chavdarov Peter *et al.* Evaluation of lentil germplasm for disease resistance to fusarium wilt (*Fusarium Oxysporum*

f.sp. lentis): Journal of Central European Agriculture, 2006, 7(1).
7. Aminot *et al*. Standard procedure for the determination of chlorophyll *a* by spectroscopic methods. International Council for the Exploration of the Sea, 2000. ISSN0903-2606.
8. Aron D. Copper enzymes isolated chloroplasts, polyphenoloxidase in *Beta vulgaris.* Plant Physiology. 1949; 24:1-15.
9. Ying Li *et al.*, Factors influencing Leaf chlorophyll content in Natural Forests at the Biome Scale. Frontiers in Ecology and Evolution, 2018, 6. DOI: 10.3389/fevo.2018.00064.
10. Heath MC. Hypersensitive response-related death, Plant Molecular Biology. 2000; 44:321-334.
11. Jones JD, Dangl JL. The plant immune system, Nature, 2006; 444:323-329.
12. Duca D *et al.*, Indole-3-acetic acid in plant-microbe interactions. Antonie Van Leeuwenhoek. 2014; 106:85-125.