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ECO-FRIENDLY MANAGEMENT OF STRIPE DISEASE OF BARLEY (*HORDEUM VULGARE* L.) BY PLANT EXTRACTS AND ANTAGONISTIC FUNGI

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ABSTRACT

A search for an environmentally safe and economically viable strategy for control of plant diseases has led to an increased use of plant based products in agriculture. To control the plant diseases, use of fungicides can impact the environment and human health. One method to eliminate these drawbacks is promoting induced protection. This study investigated the use of plants extracts and antagonistic fungi as a biological control of *Drechslera graminea* or as an inducer of protection in barley plant against the pathogen and also evaluated the possible mechanisms. In the controlled laboratory conditions leaf extracts of *Azadirachta indica*, *Ricinus communis*, *Calotropis procera, Ocimum sanctum, Lawsonia rosea, Cassia tora* and *Crysanthemum indicum* and native isolates of two fungal bioagents *Trichoderma harzianum* and *T. viride* were tested to examine their effectivity against *D. graminea*. *Trichoderma* sp. is an ecofriendly organism that does not cause any harmful and side effect on human beings and domestic animals when handled. Results demonstrated that *Azadiracta indica* followed by *Ocimum sanctum* were more effective as compare to remaining five ones against *Drechslera graminea*. Presence of plants extract seems to be affecting the normal growth of fungus. *Trichoderma* harzianum and T. viride were also effective against the fungal pathogen of stripe disease of barley. *Trichoderma harzianum* was found to be more effective than T. viride against *Drechslera Graminea.*

INTRODUCTION

Barley is one of the oldest crops in the world since it was domesticated thousands of years ago. It is a major cereal grain. Important uses include use as animal fodder, as a source of fermentable material for beer and certain distilled beverages, and as a component of various health foods. Barley stripe is disease of barley that once caused significant crop yield losses in many areas of the world. *Drechslera graminea* (Rabenh. ex Schlecht) Shoemaker (sexual *Pyrenophora graminea*) is the causal agent of barley stripe disease. Biocontrol or biological control appears as an attractive, ecofriendly and realistic approach to control plant fungal pathogens. In the present agricultural and environmental scenario it is necessary to protect economically important crops through some nonhazardous and environment friendly ways. On germination of seed, the fungus systemically infects the pre-emergent seedlings. Because infested seed is only source of inoculums, seed treatment such as plant extracts and biocontrol agents *viz. Trichoderma harzianum* and *T. viride* are an effective means of disease control. Apart from conventional fungicides, plant extracts have been found effective against a wide range of pathogens (Amadioha, 2003; Bowers and Locke, 2004). Furthermore, plant product based biofungicides are systemic, specific in action, nonphytotoxic and have poor environmental retention (Singh, 1994). Studies on the mechanisms of disease control by plant extracts /products have revealed that the biologically active constituents present in them may have either direct antimicrobial activity (Amadioha, 2000; Ansari, 1995) or induce host plants defence response resulting in reduction of disease development (Schneider and Ullrich, 1994). Another alternative eco-friendly strategy which was used is application of biocontrol agents (BCAs). In recent years there has been much success in obtaining biological control of plant pathogens by mycoparasites and antagonistic fungi (Abdelmonem and Rasmy, 2000; Sarhan and Shibly, 2003; Sarhan, 2006; Al-Chaabi *et al.*, 2007). Such properties are first of all exposed by fungi *Trichoderma* and *Gliocladium*. *Trichoderma* spp. belong to the family hypocreaceae and are the potential antagonistic fungi which prevents the crops from diseases. These strains induce plants to turn on their native defense mechanisms and control pathogens. It was found that

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antagonists *Trichoderma* spp. produced a growth-regulating factor that also increases the rate of seed germination (Windham *et al.,* 1986; Sarhan and Shibly, 2000).

MATERIALS AND METHODS

Biological control of *drechslera graminea* by plant extracts

Material

Seeds of healthy, naturally infected (moderately and heavily) and artificially inoculated with *Drechslera graminea* and their seedlings after 10, 20 and 30 days from sowing, were taken for conducting studies. Leaves of seven plants *viz. Azadirachta indica*, *Ricinus communis*, *Calotropis procera, Ocimum sanctum, Lawsonia rosea, Cassia tora* and *Crysanthemum indicum* were used for their antifungal properties.

Method

Preparation of extracts: Mature leaves of seven plants were taken and crude extracts were prepared by grinding l0 g of leaves (separately for each plant). The leaves were thoroughly washed with distilled water and fine slurry was prepared from these leaves with approximately 50 ml of distilled water using pestle and mortar. The procedure was repeated thrice and each time, the resultant slurry was filtered through four layered thick muslin cloth. Distilled water was then added to the crude extract to make its final volume to 250 ml.

Treatment of seeds: 100 seeds of each category were taken randomly and treated separately by dipping them in each of the seven aqueous plant extracts for 4 h. Treated seeds were dried in room temperature. Untreated seeds soaked in distilled water used as control.

Biological control of *drechslera graminea* by two antagonistic fungi

Material

Seeds of healthy, naturally infected (moderately and heavily) and artificially inoculated with *Drechslera graminea* and their seedlings after 10 days, 20 days and 30 days from sowing, were taken for conducting studies. Two antagonistic fungi *Trichoderma harzianum* and *T. viride* were used as a biological control agents (BCAs) for control the disease caused by *Drechslera graminea*.

Method

Preparation of spore suspension: Pure culture of *Trichoderma harzianum* was obtained from Directorate Research of Mustard Rapeseed (DRMR), Sewar, Bharatpur and *T. viride* was obtained from Adaptive Trial Research Centre (ATRC), Malikpur, Bharatpur and raised on PDA plate for seed treatment. Spore suspensions of these fungi were prepared from 12 days old sporulating cultures (2×10^5 conidia/ml) with the aid of a haemotocytometer (Sarhan, 2006). 10 ml of water was added to each 12 days old sporulating cultures plate and the suspension was diluted to 20 ml of autoclaved distilled water.

Seed treatment: One seed sample infected with *Drechslera graminea* (naturally and artificially) was used. 200 seeds per treatment (naturally infected and artificially inoculated) were taken at random and surface sterilized with 1 % Chlorine solution. Treatments were done by dipping seeds in a flask with 25 ml of prepared spore suspension ((2×10^5 conidia/ml) of *T. harzianum* and *T. viride* amended with autoclaved 0.5 % methyl cellulose (as adhesive material), separately for 4 h and were dried in room temperature. Untreated seeds soaked in distilled water were used as control.

Observation on disease incidence by Petriplate method

Treated and untreated (control) seeds were sown in petriplates (10 seeds/ petriplate) on blotter for 7 days. Observation on seed germination, seedling mortality and disease incidence were made after every 24 h intervals up to 7^{th} day.

Biochemical Estimation of primary metabolites in seedlings

The emerging seedlings were excised for the estimation of primary metabolites at 10, 20 and 30 days after sowing. Estimation of primary metabolites: Total sugars and starch were estimated by the method of Dubois *et al.* (1956). Total phenols were determined by Swain and Hillis's method (1959) and total proteins were measured according Lowry *et al.* (1951). There were three replicates of each treatment and biochemical tests were done in three replications.

Statistical Analysis: All experiments were performed in 3 different sets with each set in triplicates. The data are expressed as mean and ± SD (standard daviation). Statistical analysis of data was done by using Graph pad prism 5 statistical software in a completely randomized design. Graphs were drawn by using Microsoft Excel software.

RESULT AND DISCUSSION

Biocontrol or biological control appears as an attractive, ecofriendly and realistic approach to control plant fungal pathogens. In the present agricultural and environmental scenario it is necessary to protect economically important crops through some nonhazardous and environment friendly ways. On germination of seed, the fungus systemically infects the pre-emergent seedlings. Because infested seed is only source of inoculums, seed treatment such as plant extracts and biocontrol agents *viz. Trichoderma harzianum* and *T. viride* are an effective means of disease control.

Biocontrol by Plant Extracts

The results of present investigation showed that all seven plant extracts were inhibited the growth of leaf stripe pathogen of barley with inhibition varying from one extract to another. A significant control of stripe disease of barley was observed in the plants in which seeds treated with neem (*Azadiracta indica*) leaf extract followed by *Ocimum sanctum* leaf extract. Seeds treated with other remaining five plant leaf extract *viz. Calotropis procera, Cassia tora, Crysanthemum indicum, Lawsonia rosea* and *Ricinus communis* were showed least control over *Drechslera graminea*. Maximum severity of disease was observed in control. Seed germination (%) was also promoted by seed treatment with plant extracts. Maximum seed germination and lowest disease incidence was observed with *Azadiracta indica* seed treatment. Seedling mortality was also reduced by *Azadiracta indica* seed treatment.

Table 1. Amount of Sugars in seedling of healthy, moderately & heavily infected and artificially inoculated, seeds treated with leaf extracts of *Azadiracta indica* and *Ocimum sanctum* individually, on 10th, 20th, and 30th day of sowing

S. No.	Treatment	Category of seedlings	Amount of Sugars (mg/g f. wt.)		
			Incubation time		
			10 days	20 days	30 days
1.	Control (untreated)	Healthy	22.69 ± 0.87	23.12 ± 1.15	23.69 ± 0.24
		Moderately infected	21.15 ± 0.62	21.26 ± 0.87	21.65 ± 0.46
		Heavily infected	21.58 ± 0.29	21.45 ± 1.07	21.05 ± 0.32
		Artificially inoculated	22.26 ± 0.47	22.56 ± 0.81	22.06 ± 0.91
2.	Treated with *Azadiracta indica* extract	Healthy	22.87 ± 0.010	23.55 ± 0.020	23.99 ± 0.064
		Moderately infected	21.44 ± 0.011	21.37 ± 0.017	21.89 ± 0.005
		Heavily infected	21.87 ± 0.011	21.73 ± 0.017	21.91 ± 0.023
		Artificially inoculated	22.62 ± 0.011	22.60 ± 0.011	22.85 ± 0.011
3.	Treated with *Ocimum sanctum* extract	Healthy	22.85 ± 0.015	23.52 ± 0.026	23.97 ± 0.017
		Moderately infected	21.44 ± 0.020	21.35 ± 0.015	21.87 ± 0.015
		Heavily infected	21.86 ± 0.023	21.74 ± 0.020	21.92 ± 0.030
		Artificially inoculated	22.62 ± 0.025	22.62 ± 0.015	22.82 ± 0.020

The values indicated in the table are the mean of three replications with standard deviation (±SD)

Table 2. Amount of Starch in seedling of healthy, moderately & heavily infected and artificially inoculated seeds treated with leaf extracts of *Azadiracta indica* and *Ocimum sanctum* individually, on 10th, 20th, and 30th day of sowing

S. No.	Treatment	Category of seedlings	Amount of Starch (mg/g f. wt.)		
			Incubation time		
			10 days	20 days	30 days
1.	Control (untreated)	Healthy	30.27 ± 0.56	30.52 ± 0.63	30.09 ± 0.73
		Moderately infected	30.38 ± 0.78	30.92 ± 0.63	30.19 ± 0.55
		Heavily infected	31.12 ± 0.76	31.58 ± 0.92	30.94 ± 0.70
		Artificially inoculated	31.45 ± 1.57	31.56 ± 0.50	31.12 ± 1.55
2.	Treated with *Azadiracta indica* extract	Healthy	31.50 ± 0.020	31.79 ± 0.011	31.88 ± 0.011
		Moderately infected	30.53 ± 0.015	30.97 ± 0.025	31.16 ± 0.011
		Heavily infected	30.32 ± 0.015	30.69 ± 0.005	30.99 ± 0.005
		Artificially inoculated	31.49 ± 0.015	31.79 ± 0.015	32.01 ± 0.057
3.	Treated with *Ocimum sanctum* extract	Healthy	31.47 ± 0.011	31.75 ± 0.011	31.89 ± 0.005
		Moderately infected	30.54 ± 0.011	30.98 ± 0.011	31.13 ± 0.011
		Heavily infected	30.30 ± 0.011	30.66 ± 0.011	30.97 ± 0.011
		Artificially inoculated	31.48 ± 0.011	31.77 ± 0.011	31.99 ± 0.023

The values indicated in the table are the mean of three replications with standard deviation (±SD)

Table 3. Amount of Proteins in seedling of healthy, moderately & heavily infected and artificially inoculated seeds treated with leaf extracts of *Azadiracta indica* and *Ocimum sanctum* individually, on 10th, 20th, and 30th day of sowing

S. No.	Treatment	Category of seedlings	Amount of Proteins (mg/g f. wt)		
			Incubation time		
			10 days	20 days	30 days
1.	Control (untreated)	Healthy	32.23 ± 0.76	35.23 ± 0.94	35.65 ± 0.68
		Moderately infected	33.45 ± 0.80	36.00 ± 0.85	35.89 ± 0.85
		Heavily infected	33.06 ± 0.58	36.45 ± 0.85	36.49 ± 0.76
		Artificially inoculated	34.36 ± 0.70	35.12 ± 1.78	35.48 ± 0.62
2.	Treated with *Azadiracta indica* extract	Healthy	33.08 ± 0.011	35.87 ± 0.011	36.40 ± 0.011
		Moderately infected	34.09 ± 0.011	36.19 ± 0.010	38.30 ± 0.011
		Heavily infected	33.43 ± 0.011	36.61 ± 0.011	38.04 ± 0.011
		Artificially inoculated	34.48 ± 0.011	35.50 ± 0.011	36.43 ± 0.011
3.	Treated with *Ocimum sanctum* extract	Healthy	33.06 ± 0.023	35.82 ± 0.011	36.41 ± 0.023
		Moderately infected	34.06 ± 0.052	36.19 ± 0.010	38.28 ± 0.015
		Heavily infected	33.40 ± 0.011	36.61 ± 0.011	38.01 ± 0.011
		Artificially inoculated	34.48 ± 0.023	35.46 ± 0.023	36.52 ± 0.020

The values indicated in the table are the mean of three replications with standard deviation (±SD)

Table 4. Amount of Phenols in seedling of healthy, moderately & heavily infected and artificially inoculated seeds treated with leaf extracts of *Azadiracta indica* and *Ocimum sanctum* individually, on 10th, 20th, and 30th day of sowing

S. No.	Treatment	Category of seedlings	Amount of Phenols (mg/g f. wt.)		
			Incubation time		
			10 days	20 days	30 days
1.	Control (untreated)	Healthy	08.12 ± 0.15	08.16 ± 0.38	07.98 ± 0.19
		Moderately infected	08.05 ± 0.14	08.25 ± 0.10	08.69 ± 0.08
		Heavily infected	08.45 ± 0.27	08.45 ± 0.17	08.73 ± 0.43
		Artificially inoculated	08.16 ± 0.41	08.49 ± 0.11	08.93 ± 0.12
2.	Treated with *Azadiracta indica* extract	Healthy	07.96 ± 0.011	08.20 ± 0.011	08.34 ± 0.011
		Moderately infected	08.08 ± 0.011	08.22 ± 0.005	08.39 ± 0.011
		Heavily infected	08.38 ± 0.011	08.42 ± 0.011	08.49 ± 0.011
		Artificially inoculated	08.12 ± 0.020	08.48 ± 0.011	08.69 ± 0.011
3.	Treated with *Ocimum sanctum* extract	Healthy	07.98 ± 0.020	08.24 ± 0.011	08.37 ± 0.011
		Moderately infected	08.06 ± 0.017	08.20 ± 0.011	08.41 ± 0.011
		Heavily infected	08.33 ± 0.005	08.43 ± 0.011	08.45 ± 0.005
		Artificially inoculated	08.08 ± 0.011	08.50 ± 0.011	08.70 ± 0.011

The values indicated in the table are the mean of three replications with standard deviation (±SD)

Table 5. Amount of Sugars in seedling of healthy, moderately & heavily infected and artificially inoculated seeds treated with spore suspension of *Trichoderma harzianum* and *T. viride* individually, on 10th, 20th, and 30th day of sowing

S. No.	Treatment	Category of seedlings	Amount of Sugars (mg/g f. wt.)		
			Incubation time		
			10 days	20 days	30 days
1.	Control (untreated)	Healthy	22.69 ± 0.87	23.12 ± 1.15	23.69 ± 0.24
		Moderately infected	21.15 ± 0.62	21.26 ± 0.87	21.65 ± 0.46
		Heavily infected	21.58 ± 0.29	21.45 ± 1.07	21.05 ± 0.32
		Artificially inoculated	22.26 ± 0.47	22.56 ± 0.81	22.06 ± 0.91
2.	Treated with *Trichoderma harzianum*	Healthy	22.87 ± 0.015	23.55 ± 0.026	23.99 ± 0.017
		Moderately infected	21.44 ± 0.020	21.37 ± 0.015	21.87 ± 0.015
		Heavily infected	21.86 ± 0.023	21.74 ± 0.020	21.92 ± 0.030
		Artificially inoculated	22.62 ± 0.025	22.62 ± 0.015	22.82 ± 0.020
3.	Treated with *Trichoderma viride*	Healthy	22.83 ± 0.020	23.53 ± 0.015	23.73 ± 0.015
		Moderately infected	21.39 ± 0.005	21.38 ± 0.015	21.68 ± 0.015
		Heavily infected	21.71 ± 0.017	21.88 ± 0.011	22.05 ± 0.046
		Artificially inoculated	22.28 ± 0.565	22.63 ± 0.005	22.93 ± 0.005

The values indicated in the table are the mean of three replications with standard deviation (±SD)

Table 6. Amount of Starch in seedling of healthy, moderately & heavily infected and artificially inoculated seeds treated with spore suspension of *Trichoderma harzianum* and *T. viride* individually, on 10th, 20th, and 30th day of sowing

S. No.	Treatment	Category of seedlings	Amount of Starch (mg/g f. wt.)		
			Incubation time		
			10 days	20 days	30 days
1.	Control (untreated)	Healthy	30.27 ± 0.56	30.22 ± 0.63	32.89 ± 0.73
		Moderately infected	30.38 ± 0.78	30.92 ± 0.63	30.19 ± 0.55
		Heavily infected	31.12 ± 0.76	31.58 ± 0.92	30.94 ± 0.70
		Artificially inoculated	31.45 ± 1.57	31.56 ± 0.50	31.12 ± 1.55
2.	Treated with *Trichoderma harzianum*	Healthy	31.53 ± 0.020	31.84 ± 0.017	32.14 ± 0.011
		Moderately infected	30.61 ± 0.041	31.04 ± 0.020	31.52 ± 0.011
		Heavily infected	30.24 ± 0.017	30.64 ± 0.015	30.98 ± 0.104
		Artificially inoculated	31.51 ± 0.015	31.73 ± 0.032	31.98 ± 0.020
3.	Treated with *Trichoderma viride*	Healthy	31.50 ± 0.020	31.79 ± 0.011	31.88 ± 0.011
		Moderately infected	30.53 ± 0.015	30.97 ± 0.025	31.16 ± 0.011
		Heavily infected	30.32 ± 0.015	30.69 ± 0.005	30.99 ± 0.005
		Artificially inoculated	31.49 ± 0.015	31.79 ± 0.015	32.01 ± 0.057

The values indicated in the table are the mean of three replications with standard deviation (±SD)

Table 7. Amount of Proteins in seedling of healthy, moderately & heavily infected and artificially inoculated seeds treated with spore suspension of *Trichoderma harzianum* and *T. viride* individually, on 10th, 20th, and 30th day of sowing

S. No.	Treatment	Category of seedlings	Amount of Proteins (mg/g f. wt.)		
			Incubation time		
			10 days	20 days	30 days
1.	Control (untreated)	Healthy	34.23 ± 0.76	35.23 ± 0.94	35.65 ± 0.68
		Moderately infected	33.45 ± 0.80	36.00 ± 0.85	35.89 ± 0.85
		Heavily infected	33.06 ± 0.58	36.45 ± 0.85	36.49 ± 0.76
		Artificially inoculated	34.36 ± 0.70	35.12 ± 1.78	35.48 ± 0.62
2.	Treated with *Trichoderma harzianum*	Healthy	33.08 ± 0.034	35.85 ± 0.035	36.42 ± 0.011
		Moderately infected	34.05 ± 0.064	36.19 ± 0.010	38.30 ± 0.015
		Heavily infected	33.40 ± 0.011	36.61 ± 0.011	38.01 ± 0.011
		Artificially inoculated	34.48 ± 0.023	35.46 ± 0.023	36.52 ± 0.020
3.	Treated with *Trichoderma viride*	Healthy	33.10 ± 0.015	35.84 ± 0.020	37.00 ± 0.023
		Moderately infected	34.10 ± 0.011	36.14 ± 0.015	37.21 ± 0.011
		Heavily infected	33.38 ± 0.011	36.61 ± 0.026	37.34 ± 0.011
		Artificially inoculated	34.45 ± 0.030	35.45 ± 0.011	36.51 ± 0.011

The values indicated in the table are the mean of three replications with standard deviation (±SD)

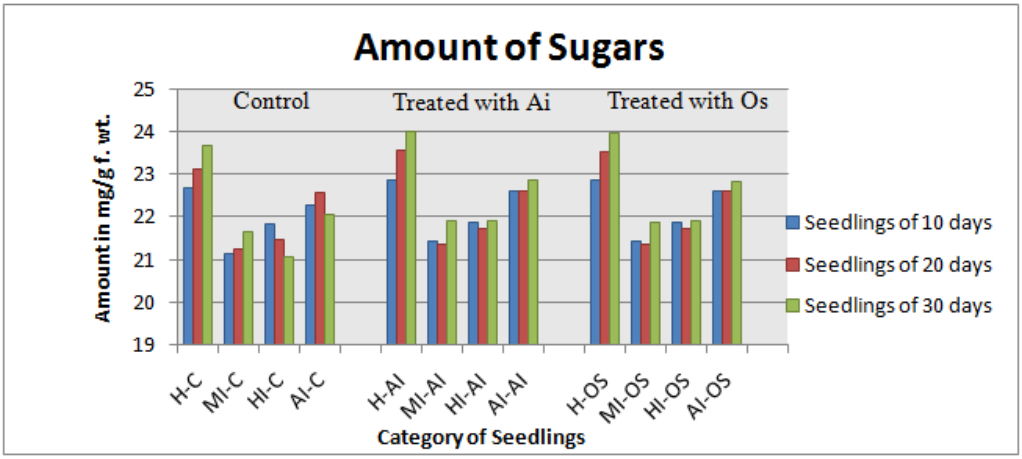
Table 8. Amount of Phenols in seedling of healthy, moderately & heavily infected and artificially inoculated seeds treated with spore suspension of *Trichoderma harzianum* and *T. viride* individually, on 10th, 20th, and 30th day of sowing

S. No.	Treatment	Category of seedlings	Amount of Phenols (mg/g f. wt.)		
			Incubation time		
			10 days	20 days	30 days
1.	Control (untreated)	Healthy	08.12 ± 0.15	08.16 ± 0.38	07.98 ± 0.19
		Moderately infected	08.05 ± 0.14	08.25 ± 0.10	08.69 ± 0.08
		Heavily infected	08.45 ± 0.27	08.45 ± 0.17	08.73 ± 0.43
		Artificially inoculated	08.16 ± 0.41	08.49 ± 0.11	08.93 ± 0.12
2.	Treated with *Trichoderma harzianum*	Healthy	07.98 ± 0.020	08.24 ± 0.020	08.37 ± 0.011
		Moderately infected	08.06 ± 0.017	08.20 ± 0.011	08.41 ± 0.011
		Heavily infected	08.38 ± 0.011	08.42 ± 0.011	08.49 ± 0.011
		Artificially inoculated	08.12 ± 0.020	08.48 ± 0.011	08.69 ± 0.011
3.	Treated with *Trichoderma viride*	Healthy	08.00 ± 0.041	08.14 ± 0.011	08.17 ± 0.011
		Moderately infected	08.11 ± 0.011	08.19 ± 0.030	08.31 ± 0.011
		Heavily infected	08.40 ± 0.011	08.40 ± 0.011	08.49 ± 0.011
		Artificially inoculated	08.13 ± 0.011	08.47 ± 0.005	08.67 ± 0.011

The values indicated in the table are the mean of three replications with standard deviation (±SD)

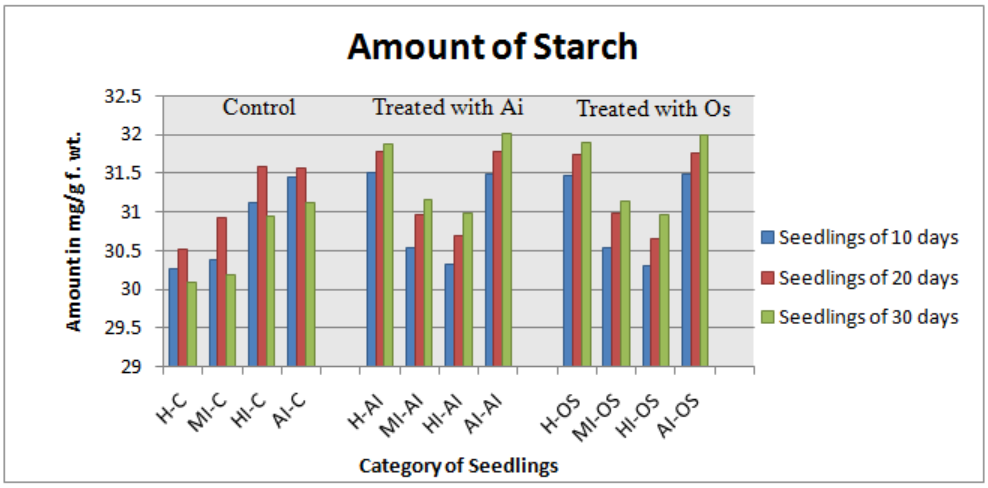
The observations thus taken indicate that all extracts probably have more or less some fungicidal properties that inhibited the growth of the pathogen. Presence of plant extracts seems to be affecting the normal growth of fungus. Results of biochemical estimations of sugars, starch, proteins and phenols revealed that sugars, starch and proteins contents were significantly higher and phenols were lower in *Azadiracta indica* and *Ocimum sanctum* leaf extract treated seedlings as compared to control in all the categories healthy, naturally infected and artificially inoculated. Thus biochemical estimations also supported that extracts of *Azadiracta indica* and *Ocimum sanctum* are more or less inhibitory to growth of mycelium of the pathogen. Treatment with these extracts led to the changes in plant metabolism. It is therefore, suggested that, protection of barley plants against *Drechslera graminea* by these two plants extract might be due to stimulation of plants natural defence response.

Erysiphe pisi but there was a reduction in the number of germlings producing multiple germ tubes. Pea plants treated with Neemazal had higher PAL activity which may be the reason for protection against the disease (Singh and Prithviraj, 1997). These results agrees with the findings of Varshney (2001). She reported that water extracts of *Azadirachta indica*, *Lantana camara*, *Pinus roxburghii*, *Tagetes erecta* and fraction of mustard oil cake inhibited the sporulation, spore germination and growth of mycelium of the leaf stripe pathogen of barley. Paul and Sharma (2002) also reported that the aqueous extract of leaves of neem (*Azadirachta indica*) provided control of leaf stripe pathogen on barley that was effective as good as fungicide bavistin. It was further observed that the treated leaves exhibited significantly high activity of enzymes phenylalanine ammonia-lyase (PAL) and tyrosine ammonia- lyase (TAL) along with rapid and distinct accumulation of fungitoxic phenolic compounds; Significant



Ai- *Azadiracta indica*, Os- *Ocimum sanctum*, H- Healthy, MI- Moderately infected, HI- Heavily infected, AI- Artificially inoculated

Figure 1. Amount of Sugars in seedling of healthy, moderately & heavily infected and artificially inoculated seeds treated with leaf extracts of *Azadiracta indica* and *Ocimum sanctum* individually, on 10th, 20th, and 30th day of sowing



Ai- *Azadiracta indica*, Os- *Ocimum sanctum*, H- Healthy, MI- Moderately infected, HI- Heavily infected, AI- Artificially inoculated

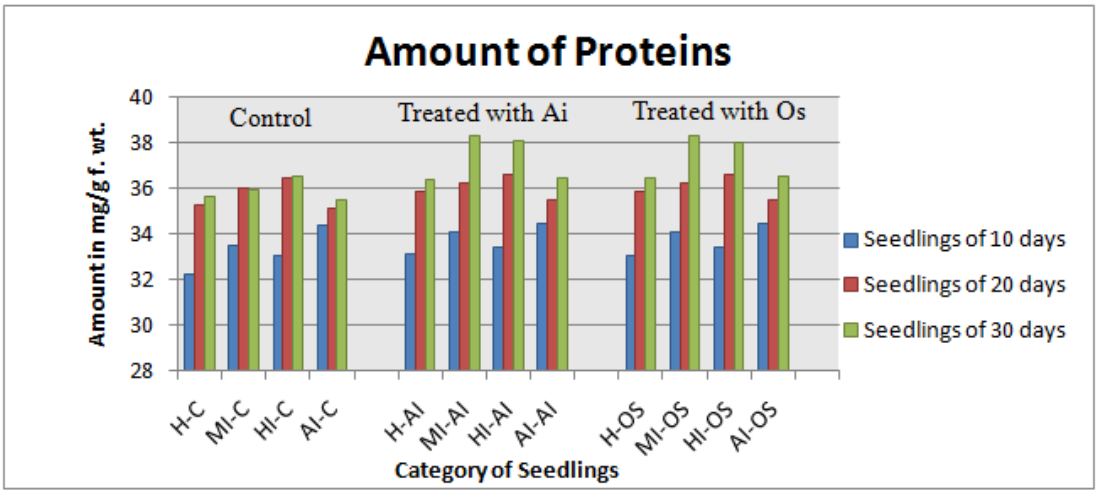
Figure 2. Amount of Starch in seedling of healthy, moderately & heavily infected and artificially inoculated seeds treated with leaf extracts of *Azadiracta indica* and *Ocimum sanctum* individually, on 10th, 20th, and 30th day of sowing

Several researchers have shown that plant extracts may control fungal plant pathogens (Parveen and Kumar, 2000; Agrios, 2005a). Antifungal proteins from *sorghum* seeds inhibited spore germination and could bring about hyphal rupture in *Fusarium moniliforme and Curvularia lunata* (Seetharaman *et al.,* 1997*)*. Neemazal, a product derived from neem did not affect the conidial germination or appressorium formation in

control of *Alternaria* leaf spot disease was observed by Guleria and Kumar (2006) in the plants treated with neem leaf extract. Maximum severity of disease was observed in control (65%), whereas, in plants treated with neem leaf extract (1:2 dilution). Bavaji *et al.* (2012) also stated that the leaf extracts of *Boswellia ovalifoliolata*, *Euphorbia trirucalli* and *Cassia tora* are effective to reduce the growth of *Alternaria alternata* at

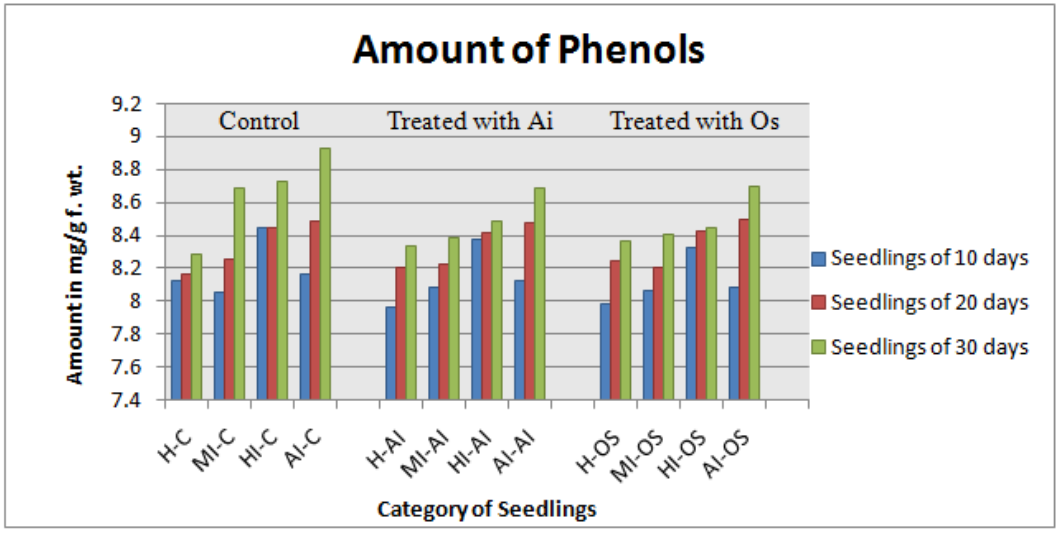
500ppm concentrations in sesame plant. Bhuvaneswari *et al*. (2012) reported that treatment of single leaf of barley seedlings with aqueous fruit extract of neem could protect the untreated and later emerging leaves of these seedlings from infection by leaf stripe pathogen. The concentration of Salicylic acid (SA) and activities of phenylalanine ammonia lyase (PAL) and peroxidase (PO) were significantly higher in untreated leaves of seedlings given a single leaf treatment with neem fruit extract. Neem fruit extract induced SAR in barley seedlings against *Drechslera graminea*.

The observations thus taken indicate that suspension of *Trichoderma harzianum* and *T. viride* are more or less inhibitory to growth of mycelium of the pathogen. These biocontrol agents act through mycoparasitism and are aggressive competitors to the pathogens through production of antibiotics thus presence of *Trichoderma* sp. affect the normal growth of fungus. Results of biochemical estimations of sugars, starch, proteins and phenols revealed that sugars, starch and proteins contents were significantly higher and phenols were lower in *Trichoderma harzianum* and *Trichoderma viride*



Ai- *Azadiracta indica*, Os- *Ocimum sanctum*, H- Healthy, MI- Moderately infected, HI- Heavily infected, AI- Artificially inoculated

Figure 3. Amount of Proteins in seedling of healthy, moderately & heavily infected and artificially inoculated seeds treated with leaf extracts of *Azadiracta indica* and *Ocimum sanctum* individually, on 10th, 20th, and 30th day of sowing



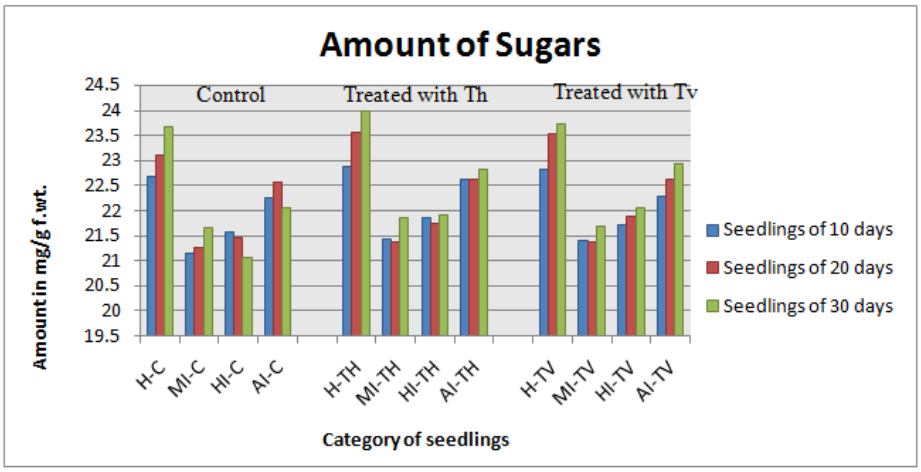
Ai- *Azadiracta indica*, Os- *Ocimum sanctum*, H- Healthy, MI- Moderately infected, HI- Heavily infected, AI- Artificially inoculated

Figure 4. Amount of Phenols in seedling of healthy, moderately & heavily infected and artificially inoculated seeds treated with leaf extracts of *Azadiracta indica* and *Ocimum sanctum* individually, on 10th, 20th, and 30th day of sowing

Biocontrol by Biological Control Agents

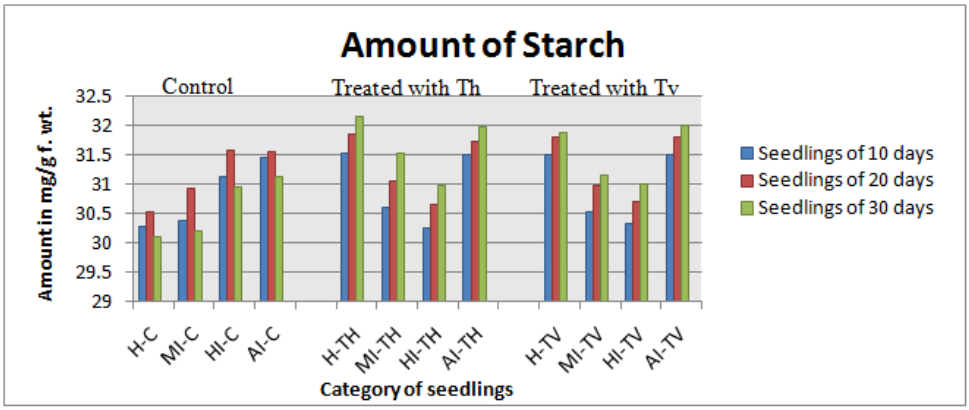
Results of present investigations revealed that *Trichoderma harzianum* followed by *T. viride* provided a significant control of stripe disease of barley. Percent seed germination was increased and disease incidence was decreased in treated seeds as compared to control. Maximum severity of disease was observed in control. Maximum germination was found in *Trichoderma harzianum* treated seed.

treated seedlings as compared to control in all the categories healthy, naturally infected and artificially inoculated. Thus biochemical estimations also supported that suspension of *Trichoderma harzianum* and *T. viride* are more or less inhibitory to growth of mycelium of the pathogen. The inhibition of *Drechslera graminea* by *Trichoderma* species could probably be due to the secretion of extracellular cell degrading enzymes such as chitinase B-1, 3- glucanase, cellulose and lectin, which help mycoparasites in the colonization of their host.



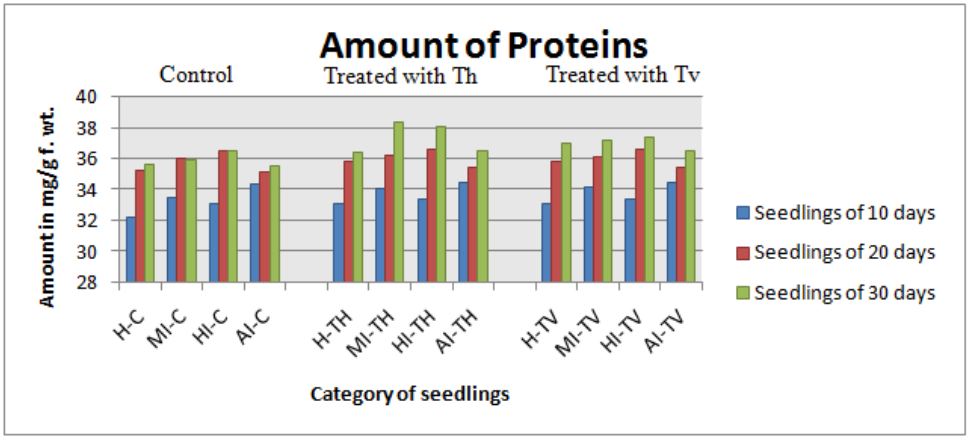
H- Healthy, MI-Moderately infected, HI-Heavily infected, AI-Artificially inoculated. C-Control, TH-Treated with *Trichoderma harzianum*, TV-Treated with *T. viride*

Figure 5. Amount of Sugars in seedling of healthy, moderately & heavily infected and artificially inoculated seeds treated with spore suspension of *Trichoderma harzianum* and *T. viride* individually, on 10th, 20th, and 30th day of sowing



H- Healthy, MI-Moderately infected, HI-Heavily infected, AI-Artificially inoculated. C-Control, TH-Treated with *Trichoderma harzianum*, TV-Treated with *T. viride*

Figure 6. Amount of Starch in seedling of healthy, moderately & heavily infected and artificially inoculated seeds treated with spore suspension of *Trichoderma harzianum* and *T. viride* individually, on 10th, 20th, and 30th day of sowing

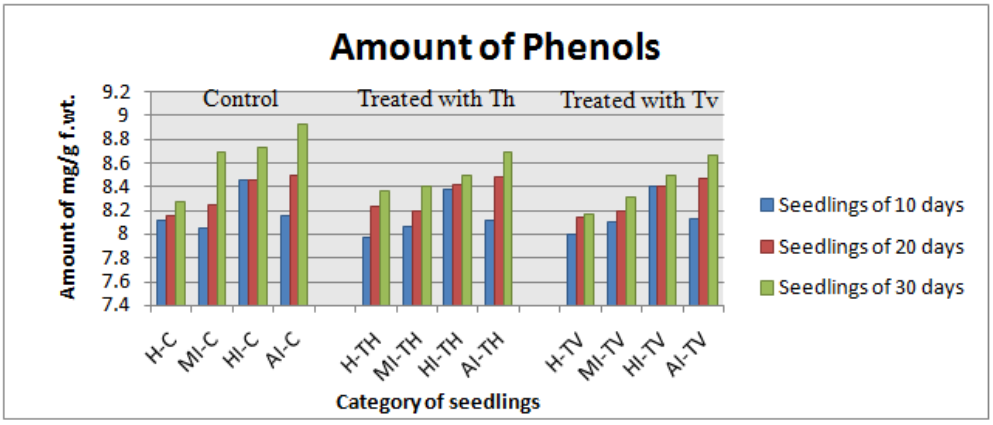


H- Healthy, MI-Moderately infected, HI-Heavily infected, AI-Artificially inoculated. C-Control, TH-Treated with *Trichoderma harzianum*, TV-Treated with *T. viride*

Figure 7. Amount of Proteins in seedling of healthy, moderately & heavily infected and artificially inoculated seeds treated with spore suspension of *Trichoderma harzianum* and *T. viride* individually, on 10th, 20th, and 30th day of sowing

The inhibition of pathogen may be also be attributed to the production of secondary metabolites by antagonists such as glioviridin, viridin and gliotoxin. (Shabir and Rubina, 2010; Kamlesh and Gurjar, 2002; Muhammad and Amusa, 2003).

Efficacy of *Trichoderma, Chaetomium*, *Trichothecium*, *Aspergillus* were tested against *Drechslera* spp. (Sudhamoy *et al*., 1999). Several research workers have been tested the different species of *Trichoderma* on various plants against any fungal pathogens.



H- Healthy, MI-Moderately infected, HI-Heavily infected, AI-Artificially inoculated. C-Control, TH-Treated with *Trichoderma harzianum*, TV-Treated with *T. viride*

Figure 8. Amount of Phenols in seedling of healthy, moderately & heavily infected and artificially inoculated seeds treated with spore suspension of *Trichoderma harzianum* and *T. viride* individually, on 10th, 20th, and 30th day of sowing

Sharma and Basandrai (2000) reported that culture filtrates of *T. harzianum, T. viride* and *G. Virens* were highly effective as seed treatment, pre and post-inoculation sprays resulting in 79.75, 74.13 and 65.44 % control over the check of Karnal Bunt pathogen of wheat, respectively. Pre, post and simultaneous inoculums of antagonists *G. virens, T. harzianum* and *T. viride* have been also reported earlier to be effective in reducing the Kernel bunt incidence of wheat (Sharma *et al*., 1996). Abdelmonem and Rasmy (2000) found that seed coating with *Trichoderma* spp. was the best biological treatment for reducing seed and seedling infections of mangrove caused by fungi and bacteria. Pant and Mukhopadhyay (2001) also evaluated the efficacy of *Trichoderma harzianum* on seed and seedling rot complex of soybean caused by *Rhizoctonia solani*, *Sclerotium rolfsii*, *Macrophomina phaseolina*. Results revealed that volatile compounds produced by *Trichoderma harzianum* significantly reduced radial growth of all pathogens. Maximum inhibition of growth of *R. solani* (9.26%), *S. rolfsii* (51.66%) and *M. phaseolina* (32.90%) was recorded with 3 days old culture of *T. harzianum.*

Kumar and Parveen (2002) also reported that seed treatment with *T. viride* completely eliminated the seed borne fungal pathogen of leaf blight *Alternaria triticina* in wheat. Singh (2008) reported that *Trichoderma harzianum* and *T. viride* formulation consistently reduce the incidence of brown spot disease of pearlmillet and increase plant vigour index. Banyal *et al.* (2008) reported that *T. viride* inhibited maximum mycelia growth of *Sclerotium rolfsii* in tomato and was significantly better than *T. harzianum* and *G. virens.* Soil application of of *T. harzianum* and *T. viride* gave 20% collar rot incidence as compare to 46.7% in control. Both the species of *Trichoderma* have already been reported to be effective against *S. rolfsii* (Das *et al*., 2000 and Dutta *et al*., 2002). Akrami *et al.* (2012) investigated the protective effects of *Trichoderma harzianum* (T-1) and *Trichoderma asperellum* (T-2) for controlling *Fusarium* rot of bean. Results indicated that prepared conidial suspensions either in water and 10% sugar solution effectively are able to reduce the colonization of the *Fusarium solani.* Prasad *et al.* (2013) evaluated the potential of *Trichoderma viride* spore suspension as biocontrol agent against *Fusarium oxysporum* and *Alternaria alternata* in legume, black gram (*Vigna mungo)* under greenhouse conditions.

Result revealed that the seed germination (%), growth (shoot and root), vigour index and disease resistance in plant samples treated with *Trichoderma viride* increased than in controls. Lipid peroxidation levels were found to be decreased in *Trichoderma viride* treated samples. Rehman *et al.* (2013) evaluated the efficacy of *Trichoderma harzianum* and *T. viride* against the pathogen of *Fusarium* wilt in chickpea. Results indicated that seed treatment with *T. viride* and *T. harzianum* reduced the wilt incidence significantly and increased the seed germination as compared to control. Inhibition of pathogens including *Fusarium oxysporum* by *Trichoderma* species could probably be due to the secretion of extracellular cell wall degrading enzymes which help mycoparasites to colonize their host. Also, inhibition of the pathogen may be attributed to the production of secondary metabolites by the antagonists (Inbar *et al*., 1994).

Conclusion

Considering the environmental hazards of chemical fungicides, the physical, the biological or, the use of herbal fungicides may be explored for the control of plant fungal pathogens. The study indicates that diluted *Azadiracta indica* (neem) and spore suspension of *Trichoderma harzianum* can provide effective biological alternatives to chemicals for control of stripe disease of barley.

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