SINCE 1988 Vol. 34 - No. I & II June - December 2021 ISSN 0970-3586

ADVANCES IN PLANT SCIENCES

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THE ACADEMY OF PLANT SCIENCES INDIA

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ISSN 0970-3586
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Eco-Friendly Management of Charcol Rot Disease of Sorghum (*Sorghum bicolor* l. monech) by Plant extracts and Antagonistic fungi

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Charcoal rot is one of the most important disease caused by *Macrophomina phaseolina* in Sorghum. An attempt was made to determine suitable control measures including biological control by plant extracts and by *Trichoderma sp, viz Trichoderma harzianum, Trichoderma viride.* In the controlled laboratory conditions leaf extracts of *Azadirachta indica, Ricinus communis, Ocimum sanctum, Lawsonia rosea, Calotropis procera, Cassia tora* and *Crysanthemum indicum* and native isolates of two fungal bioagents *Trichoderma horzionum* and *T. viride* were tested to examine their effectivity against *Macrophomina phaseolina.* Results demonstrated that out of the seven plant species screened, the leaves extract of *Azadirachta indica* showed the highest inhibition which was followed by *Ocimum sanctum* against *Macrophomina phaseolina. Trichoderma harzianum* was found to be more effective than *T. viride* against *Macrophomina phaseolina.*

Keywords : Sorghum, *Macrophomina phaseolina,* Plant extract, *Azadirachta indica, Ocimum sanctum, Trichoderma harzianum, Trichoderma viride.*

INTRODUCTION

Sorghum bicolor (L.) Moench commonly known as "Jowar" is the most important Rabi and Kharif crop of India belonging to the family "Poaceae". It is among one of the four major cereal crop of the world, the other three being wheat, rice and maize.

Charcoal rot of sorghum is caused by the fungus *Macrophomina phaseolina* (Tassi) Goid. It is a major disease in dry regions. In India, the disease causes significant yield loss in post-rainy (Rabi) sorghum (*Sorghum bicolor*) occupying more than 5 million ha in the states of Maharashtra, Karnataka and Andhra Pradesh. *M. phaseolina* is widespread soil-borne pathogen infects a widehost range, great longevity with high competitive saprophytic potency (Su *et al.* 2001). Infection with charcoal rot was stimulated by abiotic factors such as drought stress and light-textured soil and/or biotic factors like stress associated with host reproduction (Mihail 1989). *M. phaseolina* infection leads to rotting of roots followed by rotting of stalks, resulting in lodging of the plant at later stages. Charcoal rot can cause 100% lodging, and up to 50% loss in grain yield under conditions favouring disease incidence (Mughogho and Pande, 1984), besides adversely affecting the quality of stover (dry fodder).

Biocontrol or biological control appears as an attractive, ecofriendly and realistic approach to control plant fungal pathogens while chemical fungicides may lead to the resistant varieties and also hazardous for environment. The present study aimed to provide empirical evidence of the efficacy of fungal isolates of *Trichoderma* spp. and plant extracts as biological control agents against charcoal rot in sorghum (*Sorghum bicolor*). Also, plant extracts are among the biological control agents that directly affect the plant pathogens and can induce resistance in plants against phytopathogens (Mishra and Raja 1999). Sclerotial germination of *M. phaseolina* was completely inhibited by garlic bulb extract, followed by tulsi leaf extract (Christdhas henry *et al.*, 2019). Recently, plant extracts have gained considerable attention as alternative options to synthetic fungicides and efforts have been made to utilize these extracts in the control strategies against plant diseases (Elsharkawy and El-Sawy 2015; Elkhwaga *et al.* 2018).

MATERIAL AND METHODS

Biological control of *Macrophomina phaseolina* by plant extracts

Material : Seeds of healthy, naturally infected (moderately and heavily) and artificially inoculated with *Macrophomina phaseolina* 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 *Chrysanthemum indicum* were used for their antifungal properties.

Method :

Preparation of extracts: Fresh and healthy leaves were collected and then washed with distilled water and dried in shade. The leaves were used to make a paste with distilled water (1:1, w/v) by using mixer/grinder. Powder was mixed with distilled water and left overnight to allow the constituents to get dissolved in water. Thereafter, mixture was squeezed out and extract was collected in sterile glass.

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 *Macrophomina phaseolina* by two antagonistic fungi

Material : Seeds of healthy, naturally infected (moderately and heavily) and artiflcielly inoculated with *Macrophomina phasealina* and their seedlings after 10 days, 20 days and 30 days from sowing were taken for conducting studies. Pure culture of two antagonistic fungi *Trichoderma harzianum* and *T. viride* which was obtained from MTCC (Microbial type culture collection) Chandigarh were used as a biological control agents for control the disease caused by *Macrophomina phaseolina*.

Method

Dual culture technique: Both the *T. harzianum* and *T. viride* isolates were screened individually against *M. phaseolina* by employing the dual culture technique (Asran-Amal *et al.* 2010). The *Trichoderma* spp. isolates and *M. phaseolina* were cultured, separately on PDA medium for 7 days at 25±1°C. Four day old *Trichoderma* spp. cultures were inoculated at the opposite side of the petri dish and the plates were incubated at 25±1°C for 6 days. The distance between discs was approx, 5 cm. There were four replicates for each treatment and the experiment was repeated three times.

Preparation of spore suspension: Pure culture of *Trichoderma harzianum* raised on PDA plate for seed treatment. Spore suspension of these fungi were prepared from 122 days old sporulating cultures (2 x 105 conidia/ml) with the aid of 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 v *Macrophomina phaseolina* was used 200 seeds treatment (naturally infected and artificially inoculated) w taken at random and surface sterilized with 1% Chlo solution. Treatments were done by dipping seeds in a fi with 25 ml of prepared spore suspension of *Trichode harzianum* and *T. viride* amended with autoclaved 0. methyl cellulose (as adhesive material), separately for and were dried in room temperature. Untreated seeds soa in distilled water were used as control.

Observation on disease incidence by petriplate metho

Treated and untreated seeds (control) were grow petriplates (10 seeds/petriplate) on blotter for 7 da Observation on seed germination, seedling mortality disease incidence were made after every 24 h intervals u 7th day.

Biochemical Estimation of primary metabolites in seedli

The emerging seedlings were excised for estimation of primary metabolites at 10, 20 and 30 days a sowing.

Estimation of primary metabolites : Total sugars and st were estimated by the method of Dubois *et al* (1956). T phenols were determined by Swain and Hillis's met (1959) and total proteins were measured according Lowr *al.* (1951). There were three replicates of each treatment biochemical tests were done in three replications.

RESULTS AND DISCUSSION

Biocontrol appears as an ecofriendly and real approach to control plant pathogens. Chemical control of disease is difficult, hazardous for enviornment economically not affordable for low income small farm Biological control involves the use of plant extracts biological organisms to control the pathogens and disea The exploitation of plant products and biocontrol agents the management of plant diseases have achieved gre significance due to its readily available nature, antimicrc activity, easy biodegradability, non phytotoxicity.

Biocontrol by plant extracts : In the present investiga seven plant extracts were tested against *Macrophon phaseolina*, the causal agent of charcol rot diseas sorghum. *In vitro* studies indicated the two leaf extra *Azadirachta indica* and *Ocimum sanctum* exerted the hi inhibition to mycelial growth. Seeds treated with o remaining five plant leaf extract *i.e. Calotropis proc Chrysanthemum indicum, Ricinus communis, Cassia* and *Lawsonia rosea* were showed least control *Macrophomino phaseolina*. The plant extracts contains va of chemical constituents and secondary metabolites w might cause deleterious or inhibitory effect on microorganisms. Results of biochemical estimation sugars, starch, proteins and phenols revealed that su starch and protein contents were significantly higher

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

S.No.	Treatment	Category of seedlings	Amount of Sugars (mg/g wt.) Incubation time		
			10 days	20 days	30 days
1.	Control (untreated)	Healthy	1.69	1.72	1.84
		Moderately infected	1.72	1.76	1.82
		Heavily infected	1.70	1.69	1.69
		Artificially inocuated	1.68	1.65	1.48
2.	Treated with *Azadirachta indica* extract	Healthy	1.87	1.90	1.94
		Moderately infected	1.92	2.02	2.05
		Heavily infected	1.86	1.84	1.79
		Artificially inocuated	1.69	1.82	1.78
3.	Treated with *Ocimum sanctum* extract	Healthy	1.82	1.86	1.89
		Moderately infected	1.92	1.99	2.03
		Heavily infected	1.89	1.84	1.80
		Artificially inoculated	1.69	1.67	1.56

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

Table 2 : Amount of Sugars in seedling of healthy, moderately & heavily infected and artificially inoculated, seeds treated with leaf extracts of *Acadirachta* and *Ocimum sanctum* individually, on 10th, 20th and 30th days of sowing

S.No.	Treatment	Category of seedlings	Amount of Sugars (mg/g wt.) Incubation time		
			10 days	20 days	30 days
1.	Control (untreated)	Healthy	1.52	1.59	1.64
		Moderately infected	1.36	1.58	1.49
		Heavily infected	1.48	1.45	1.42
		Artificially inocuated	1.56	1.45	1.38
2.	Treated with *Azadirachta indica* extract	Healthy	1.74	1.82	1.89
		Moderately infected	1.69	1.73	1.62
		Heavily infected	1.55	1.52	1.49
		Artificially inocuated	1.75	1.68	1.65
3.	Treated with *Ocimum sanctum* extract	Healthy	1.62	1.79	1.83
		Moderately infected	1.60	1.68	1.62
		Heavily infected	1.58	1.52	1.46
		Artificially inoculated	1.76	1.68	1.62

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

phenols were lower in *Azadirachta indica* and *Ocimum sanctum* leaf extract treated seedlings as compared to control in all the categories healthy, naturally infected and artificially inoculated. The amount of phenol were not so much significantly increased as it was suggested that pathogen was inhibited by phenolic compounds but when pathogen is successful in causing the disease then it is not allowed the phenols to increased significantly. These result are in agreement with Janci *et al.*, (2014). The results of Sugar, Starch and Protein also supported that due to presence of plant extracts the growth of fungal mycelia decreases due to which the utilization of sugars by the fungi and hydrolysis of protein by fungal proteolytic enzymes also decreases. A similar result has been reported by Dubey and kumar (2003) that Azadirachtin (30 ppm) as effective as the fungicide Mancozeb after 72 h of treatment. Padmodaya and Reddy (1999) Suprakash *et al.*, (2012), Bhatnagar *et al.*, (2013) Dabbas *et al.*, (2012), Chakraborthy et al., (2009) and Dwivedi *et al.*, (2012) studied the effect of six organic amendments including Neem cake and Eucalyptus dry leaves for their efficacy against seedling disease in tomato caused by *Fusarium oxysporium* under pot condition . Ogbebor *et al.* (2007) reported that extracts of *Ocimum basilicum* L. and *Allium sativum* L. exhibited total inhibitory effects on the mycelial growth of *Colletotrichum gloeosporioides*.

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

S.No.	Treatment	Category of seedlings	Amount of Sugars (mg/g wt.) Incubation time		
			10 days	20 days	30 days
1.	Control (untreated)	Healthy	13.12	13.24	13.46
		Moderately infected	12.97	12.80	12.79
		Heavily infected	12.92	12.68	12.62
		Artificially inocuated	13.023	12.98	12.76
2.	Treated with *Azadirachta indica* extract	Healthy	13.32	13.58	13.78
		Moderately infected	13.18	13.09	12.83
		Heavily infected	13.06	12.98	12.91
		Artificially inocuated	13.12	13.06	12.83
3.	Treated with *Ocimum sanctum* extract	Healthy	13.26	13.46	13.62
		Moderately infected	13.03	12.88	12.56
		Heavily infected	12.97	12.84	12.79
		Artificially inoculated	13.09	13.02	12.79

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 days of sowing

S.No.	Treatment	Category of seedlings	Amount of Sugars (mg/g wt.) Incubation time		
			10 days	20 days	30 days
1.	Control (untreated)	Healthy	1.45	1.62	1.84
		Moderately infected	1.59	1.71	1.83
		Heavily infected	1.78	1.89	2.03
		Artificially inocuated	1.68	1.76	1.94
2.	Treated with *Azadirachta indica* extract	Healthy	1.32	1.58	1.78
		Moderately infected	1.47	1.63	1.72
		Heavily infected	1.56	1.76	1.92
		Artificially inocuated	1.64	1.68	1.86
3.	Treated with *Ocimum sanctum* extract	Healthy	1.36	1.66	1.74
		Moderately infected	1.52	1.74	1.83
		Heavily infected	1.63	1.88	1.92
		Artificially inoculated	1.64	1.72	1.88

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

Biocontrol by Biological Control Agents : Result of present investigation showed that *Trichoderma harzianum* followed by *T. viride* provided control over charcoal rot disease of sorghum. Percent of seed germination was increased and of disease incidence was decreased in treated seeds as compared to control. Maximum seed germination and minimurTt disease incidence was observed In *Trichoderma harzianum.*

Effect of *Trichoderma* isolates on mycelial growth of *Macrophomina phaseolina in vitro* : The comparison of the study obtained from the dual culture revealed that *Trichoderma* sps. inhibited the mycelial growth of Macrophomina phaseolina. The highest level of inhibition belonged to *Trichoderma harzanium* and then *Trichoderma viride* show less inhibition then *T. harzianum*. The inhibition shown by the antagonists may be due to release of antibiotic or antibiotic like substances or hyphal parasitism which results in direct inhibition of growth of the pathogen by disintegrating the hyphal wall resulting in the penetration, absorption and lysis of the mycelium. Action of *Trichoderma* sp. leads to control root microflora, removing toxic metabolites and provides resistance against stress (Ezzi & Lynch, 2002). Antifungal activities of various bio-agents like

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 days of sowing

S.No.	Treatment	Category of seedlings	Amount of Sugars (mg/g wt.) Incubation time		
			10 days	20 days	30 days
1.	Control (untreated)	Healthy	1.69	1.72	1.84
		Moderately infected	1.72	1.76	1.82
		Heavily infected	1.70	1.69	1.63
		Artificially inocuated	1.58	1.65	1.48
2.	Treated with *Azadirachta indica* extract	Healthy	2.16	2.22	2.34
		Moderately infected	2.16	2.26	2.35
		Heavily infected	2.03	1.98	1.82
		Artificially inocuated	2.06	2.06	1.98
3.	Treated with *Ocimum sanctum* extract	Healthy	1.98	2.12	2.18
		Moderately infected	2.04	2.09	2.24
		Heavily infected	1.98	1.92	1.78
		Artificially inoculated	2.02	1.97	1.86

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 days of sowing

S.No.	Treatment	Category of seedlings	Amount of Sugars (mg/g wt.) Incubation time		
			10 days	20 days	30 days
1.	Control (untreated)	Healthy	1.52	1.59	1.64
		Moderately infected	1.36	1.57	1.49
		Heavily infected	1.48	1.45	1.22
		Artificially inocuated	1.56	1.45	1.38
2.	Treated with *Azadirachta indica* extract	Healthy	1.68	1.85	1.97
		Moderately infected	1.55	1.75	1.62
		Heavily infected	1.48	1.47	1.36
		Artificially inocuated	1.92	1.82	1.56
3.	Treated with *Ocimum sanctum* extract	Healthy	1.67	1.72	1.89
		Moderately infected	1.52	1.78	1.63
		Heavily infected	1.45	1.42	1.37
		Artificially inoculated	1.78	1.68	1.56

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

Rhizobium meliloti, *Aspergillus niger* and *Trichoderma harzianum* was tested against root rot fungi *viz. M. phaseolina*, *Rhizoctonia solani* and *Fusarium* sp. All tested agents were found effective against root rot fungi. Maximum reduction of infection was reported to produce by *Trichoderma* sp (Dawar *et al.*, 2008).

Biochemical estimation : Results of biochemical estimations of sugars, starch, proteins and phenols revealed that sugar, starch and proteins were significantly higher and phenols were lower in *Trichoderma harzianum* and *Trichoderma viride*. The reason may be attributed to increase in nutrient uptake and increased in photosynthetic rates that leads to increase in reducing sugars and soluble proteins. Similar results were observed by (K.P. *et. al*, 2011) who reported increased soluble sugar content and soluble proteins in a medicinal plant Niqella sativa.

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

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 days of sowing

S.No.	Treatment	Category of seedlings	Amount of Sugars (mg/g wt.) Incubation time		
			10 days	20 days	30 days
1.	Control (untreated)	Healthy	13.12	13.24	13.48
		Moderately infected	12.97	12.80	12.79
		Heavily infected	12.92	12.68	12.82
		Artificially inocuated	13.02	12.98	12.76
2.	Treated with *Azadirachta indica* extract	Healthy	13.22	13.36	13.52
		Moderately infected	13.13	13.09	12.97
		Heavily infected	13.02	12.97	12.89
		Artificially inocuated	13.16	13.06	12.98
3.	Treated with *Ocimum sanctum* extract	Healthy	13.20	13.37	13.49
		Moderately infected	13.09	13.07	12.93
		Heavily infected	13.05	12.98	12.87
		Artificially inoculated	13.16	13.02	12.94

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 days of sowing

S.No.	Treatment	Category of seedlings	Amount of Sugars (mg/g wt.) Incubation time		
			10 days	20 days	30 days
1.	Control (untreated)	Healthy	1.45	1.62	1.84
		Moderately infected	1.59	1.71	1.83
		Heavily infected	1.78	1.89	2.03
		Artificially inocuated	1.68	1.76	1.94
2.	Treated with *Azadirachta indica* extract	Healthy	1.36	1.62	1.79
		Moderately infected	1.52	1.64	1.72
		Heavily infected	1.61	1.75	1.91
		Artificially inocuated	1.59	1.63	1.72
3.	Treated with *Ocimum sanctum* extract	Healthy	1.41	1.68	1.72
		Moderately infected	1.50	1.72	1.79
		Heavily infected	1.68	1.79	1.85
		Artificially inoculated	1.57	1.62	1.81

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

pathogens the study indicates that diluted *Azadirachta indica* and spore suspension of *Trichoderma harzianum* can provide effective biological alternatives to chemicals for contro of charcoal rot disease of Sorghum

ACKNOWLEDGEMENT

Authors are grateful to CSIR, New Delhi for providing funds and are also grateful to the Head, Department of Botany, Govt. M.S.J.P.G. College, Bharatpur, India, for al the facilities to carry out the work.

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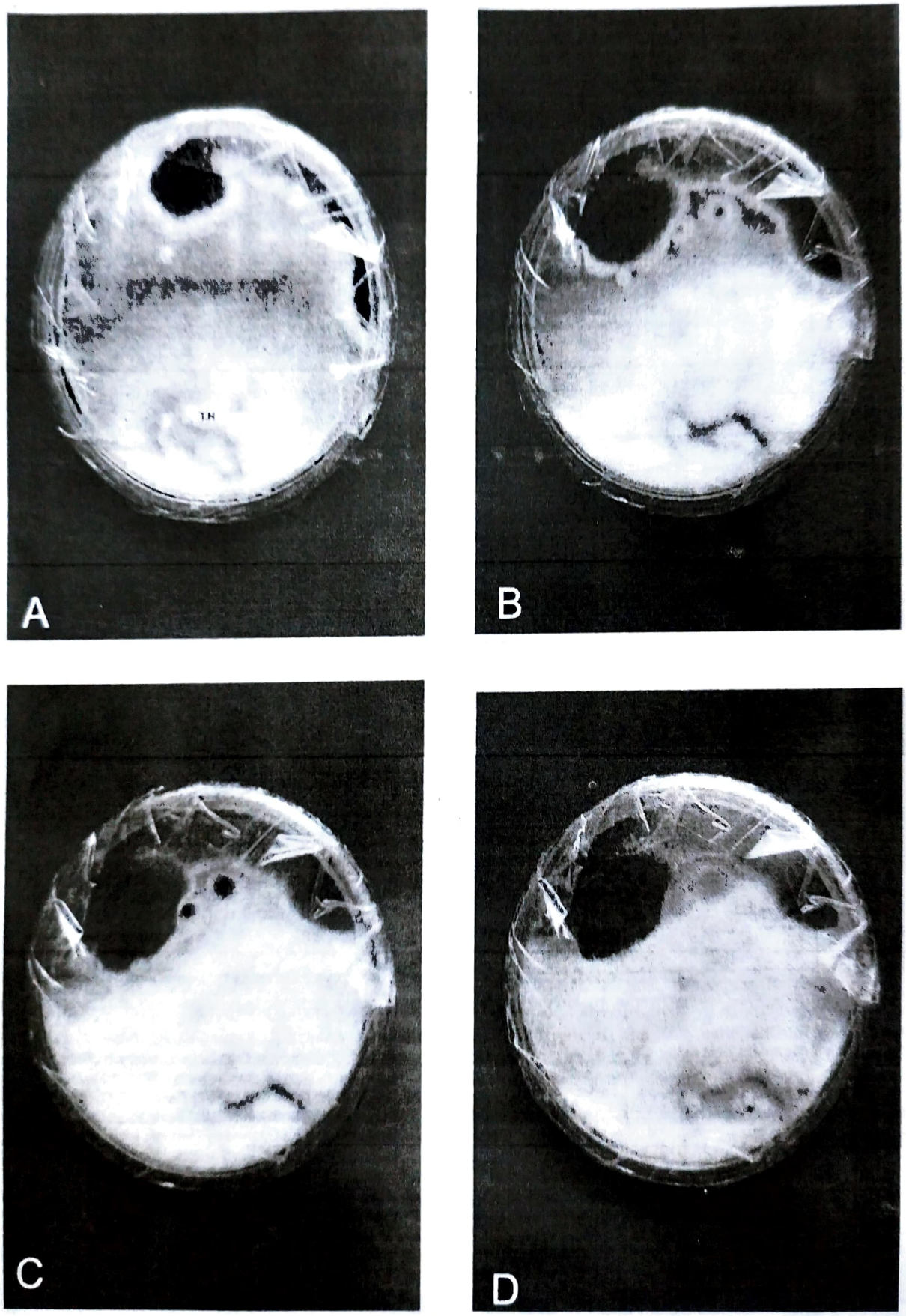
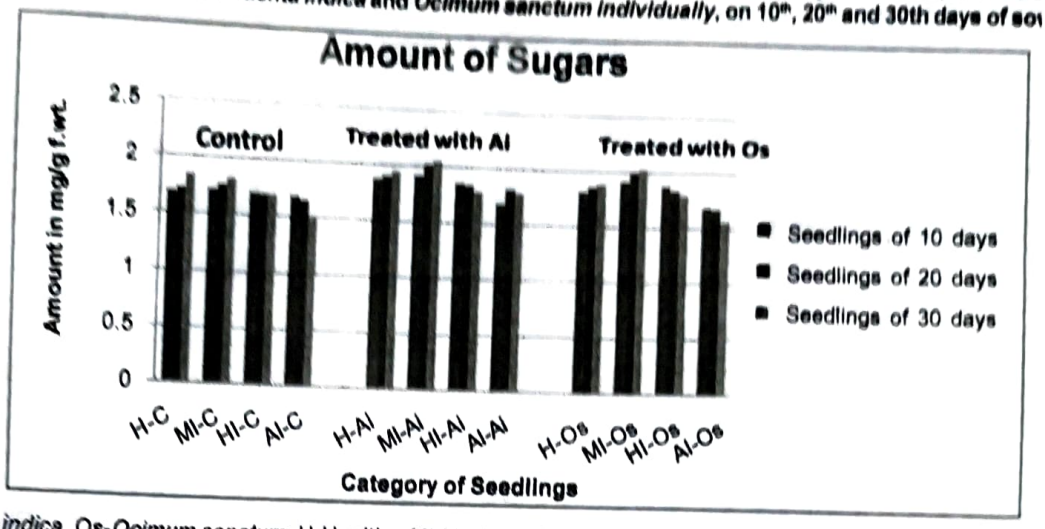


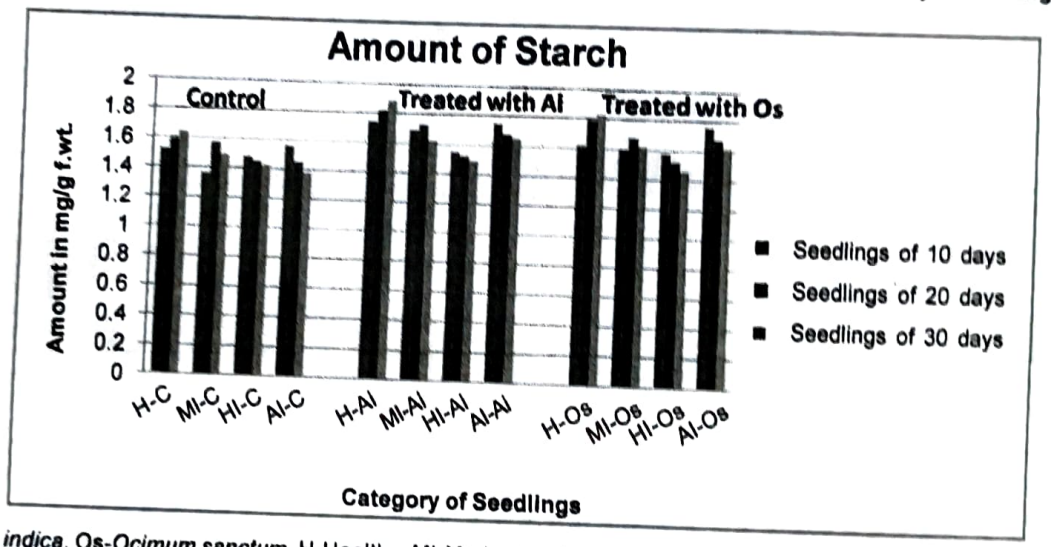
Antagonism to *Trichoderma harzianum* (T.H.) against *Macrophomina phaseoline* (M.P.)

Figure 1 : Amount of Sugars in seedling of healthy, moderately & heavily infected and artificially inoculated, seeds tre with leaf extracts of *Azadirachta indica* and *Ocimum sanctum* individually, on 10th, 20th and 30th days of so



Al-*Azadirachta indica*, Os-*Ocimum sanctum*, H-Healthy, MI-Moderately infected, HI-Heavily infected, Al-artificially infec

Figure 2 : Amount of Starch in seedling of healthy, moderately & heavily infected and artificially inoculated, seeds trea with leaf extracts of *Azadirachta* and *Ocimum sanctum* individually, on 10th, 20th and 30th days of sowing



Al-*Azadirachta indica*, Os-*Ocimum sanctum*, H-Healthy, MI-Moderately infected, HI-Heavily infected, Al-artificially infecte

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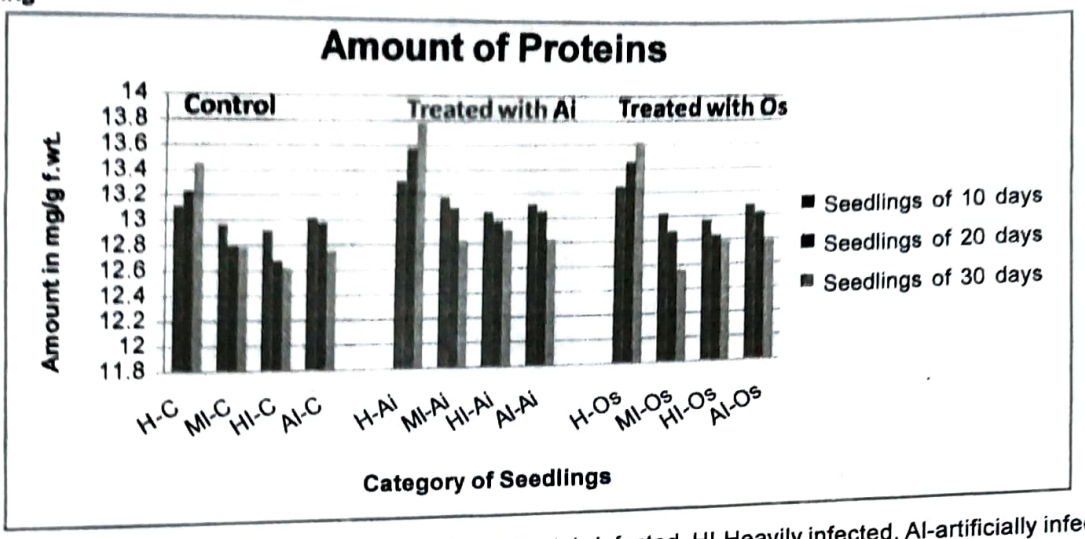
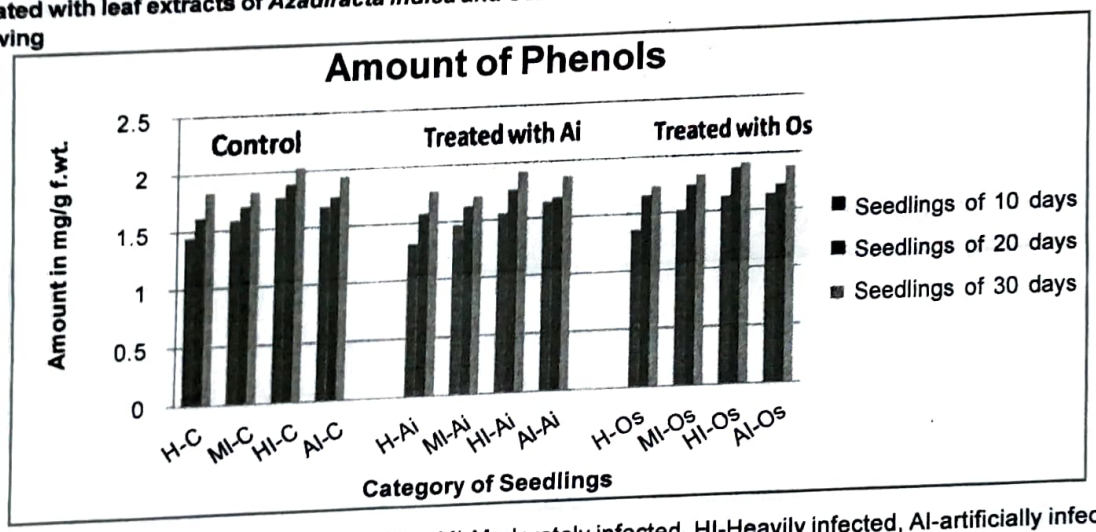
Figure 3 : Amount of Proteins in seedling of healthy, moderately & heavily infected and artificially inoculated, seeds treated with leaf extracts of *Azadirachta indica* and *Ocimum sanctum* individually, on 10th, 20th and 30th days of sowing



Ai-*Azadirachta indica*, Os-*Ocimum sanctum*, H-Healthy, MI-Moderately infected, HI-Heavily infected, AI-artificially infected

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 days of sowing



Ai-*Azadirachta indica*, Os-*Ocimum sanctum*, H-Healthy, MI-Moderately infected, HI-Heavily infected, AI-artificially infected

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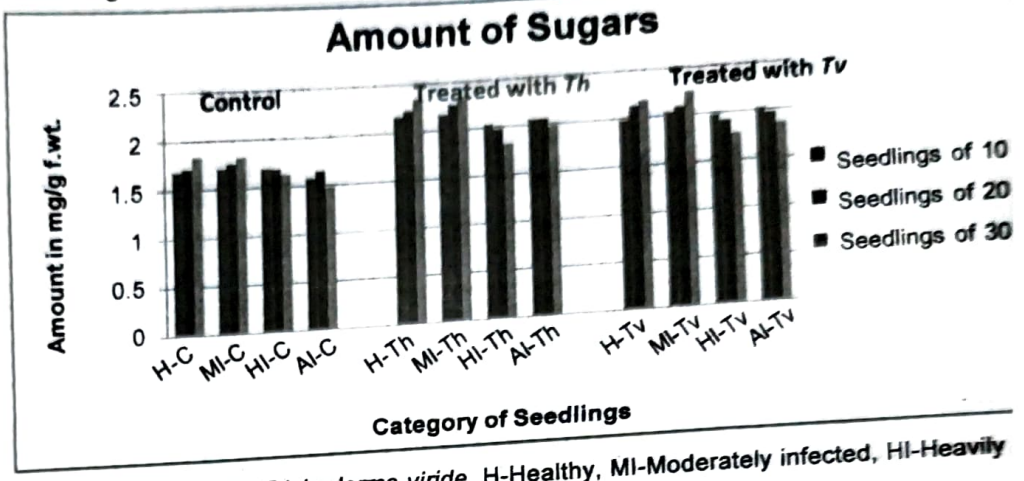
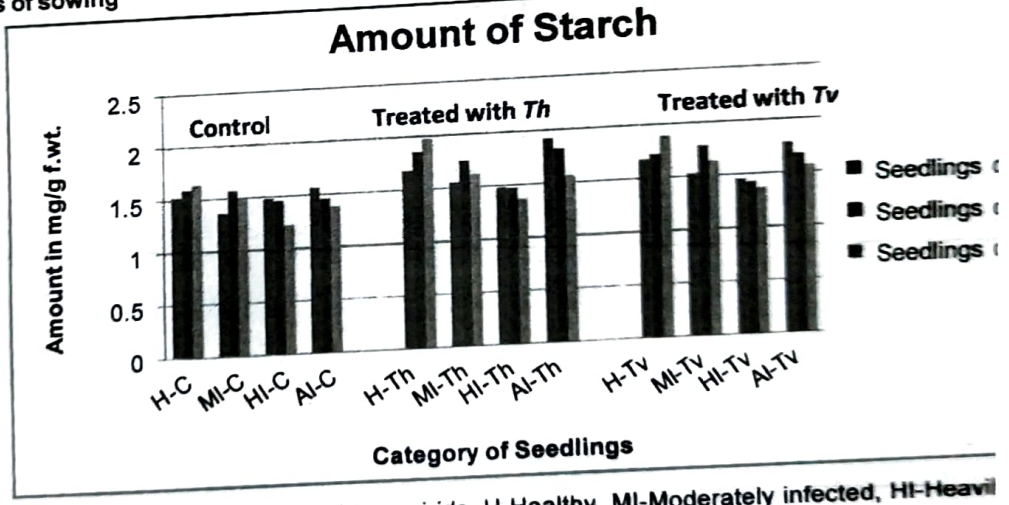
Figure 5 : Amount of Sugars in seedling of healthy, moderately & heavily infected and artificially inoculated, with spore suspension of *Trichoderma harzianum* and *Trichoderma viride* individually, on 10th days of sowing



Th-*Trichoderma harzianum*, Tv-*Trichoderma viride*, H-Healthy, MI-Moderately infected, HI-Heavily
AI-artificially infected

Figure 6 : Amount of Starch in seedling of healthy, moderately & heavily infected and artificially inoculated with spore suspension of *Trichoderma harzianum* and *Trichoderma viride* individually, on 10th days of sowing



Th-*Trichoderma harzianum*, Tv-*Trichoderma viride*, H-Healthy, MI-Moderately infected, HI-Heavil
AI-artificially infected

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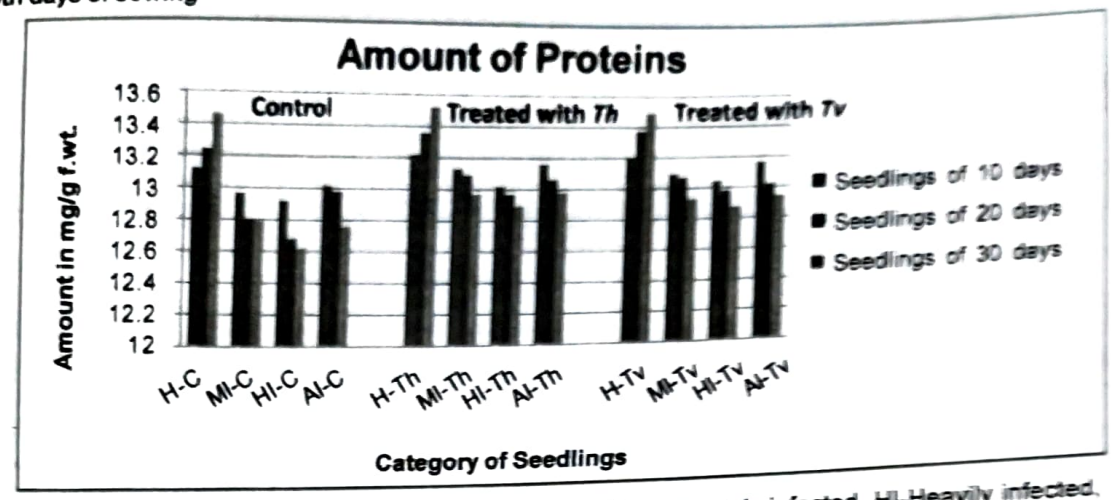
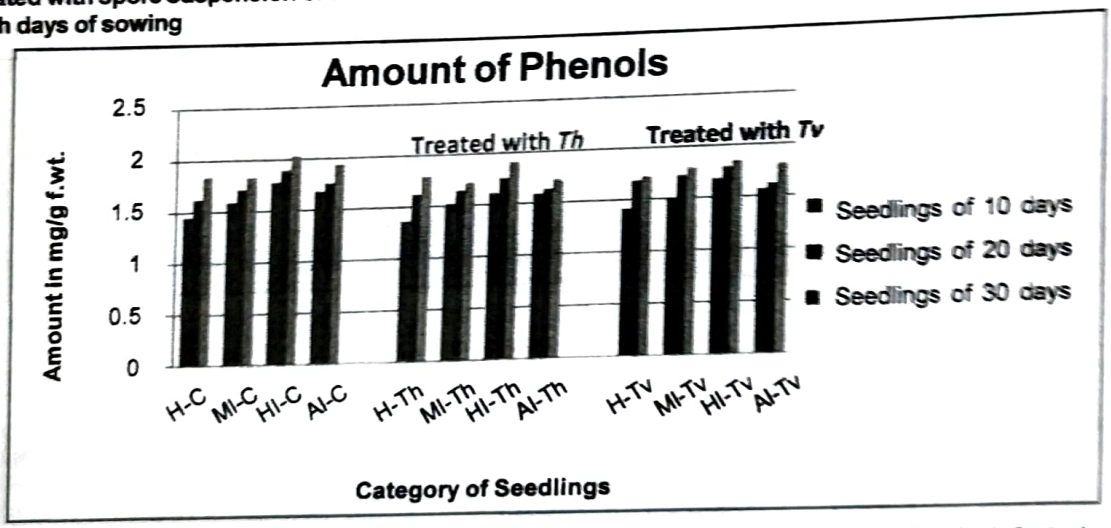
Amount of Proteins in seedling of healthy, moderately & heavily infected and artificially inoculated, seeds treated with spore suspension of *Trichoderma harzianum* and *Trichoderma viride* individually, on 10th, 20th and 30th days of sowing



ichoderma harzianum, Tv-*Trichoderma viride*, H-Healthy, MI-Moderately infected, HI-Heavily infected, AI-artificially infected

Amount of Phenols in seedling of healthy, moderately & heavily infected and artificially inoculated, seeds reated with spore suspension of *Trichoderma harzianum* and *Trichoderm viride* individually, on 10th, 20th and 30th days of sowing



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