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Full Length Research Paper

Study of the indol 3- acetic acid (IAA) induced inhibition of growth and sporulation of *Fusarium oxysporum* F. sp. *lentis* as the causal organism of wilt disease of lentil

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The wilt disease in lentil crop is a wide spread and recurrent phenomenon. Control of the disease by current technique is not sufficient. To investigate the suitable control measures by bio-molecules is highly desired. Bio-molecules are non-polluting self metabolized and biologically regulated compounds have well defined physiological action without interfering with other actions in the host plant. Inhibitory role of growth hormones and growth regulators have been indicated by many fungi. Inhibitory action of indol 3- acetic acid (IAA) on mycelial growth and sporulation of *Fusarium oxysporum* Schlecht. F. sp. *lentis* (Vasudeva and Srinivasan) as the causal organism of wilt disease of lentil (*Lens esculenta* Moench.) under *in vitro* culture have been carried out. The result indicates that mycelia growth was gradually inhibited with increase in concentration of IAA from 0.1 to 1.0 ppm. Further increase in concentration from 1.0 to 10.0 ppm, however, showed increase in growth. Sporulation of macroconidia and microconidia also reduced in number with increase in concentration from 0.1 to 1.0 ppm, though the number remained more than that of the control. Further increase in concentration from 1.0 to 10.0 ppm sporulation of both the spores increased. Sporulation of chlamydospores increased with increase in concentration from 0.1 to 1.0, thereafter inhibition of sporulation was recorded with increase in concentration from 1.0 to 10.0 ppm of IAA. This result indicated the inhibitory action of IAA for mycelia growth but sporulation was not completely inhibited.

Key words: Wilt disease, lentil wilt, *Fusarium oxysporum* f. sp. *lentis*, Indol 3- acetic acid.

INTRODUCTION

The fungal kingdom constitutes highly versatile group of eukaryotic carbon heterotrophic organisms that have successfully occupy most of the natural habitat (Knogge, 1996). Most of the known fungal species are strictly saprophytic, though less than ten percent of the 10×10^5 known fungal species are able to colonize plants. Even a smaller fraction of these are capable of causing diseases. Plant parasitic fungi have conquered the living plant as an abundant source of nutrients (Knogge, 1996; Agrios, 1997). Mendgen et al. (1996) observed different levels of specialization in plant fungal interactions.

Fungal wilt disease occurred in wide variety of economically important crops (Scheffer, 1991) and it is a major limiting factor in production of many agricultural and horticultural crops including lentil (*Lens esculenta* Moench. syn *L*. *culinaris* Medik.) (Erking and Bayaa, 1996).

Wilt of lentil is one of the major constraints in production of lentil in countries of Asia, Europe, Africa, Middle East and America. Management of Fusarium wilt is achieved mainly through chemical soil fumigation and resistant cultivars. The broad spectrum biocides such as methyl bromide used to fumigate soil before planting are damaging the environment (Groenevald, 2005). However, the most effective and environmentally safe method of control is the use of resistant cultivars (Fravel, 2003). Unfortunately, resistant breeding can be very difficult when no dominant gene is known (Buddenhagen and

Richards, 1988). To complicate the matter further, new races of the pathogen can develop which might overcome the resistance. Now there is an increasing interest among plant scientists and agricultural pathologists to evaluate potential biological molecules having antagonistic physiological actions on the fungal growth and reproduction with aim to control the disease. The effect of plant hormones and growth regulators has been found with pronounced effects on growth and reproduction (Jimenze-Diaze et al., 1993; Went, 1957). Fungi are also regulated by such phytohormones. Many fungal genera such as *Phytophthora, Cleviceps, Fusarium*, etc., have shown reduced growth and sporulation under controlled *in vitro* condition. Among several hormones, indol 3 - acetic acid (IAA) is considered as the most common growth regulator in all the plant species (Jimenze-Diaze et al., 1993; Went, 1957; Sachs, 1965). The effect of different concentrations of IAA (Mol.wt.=175.20 g/mol) on mycelial accumulation, sporulation of the three spore forms and their size, have been studied in the artificial culture of *Fusarium oxysporum* f. sp. *lentis* to evaluate the inhibitory action of IAA.

MATERIALS AND METHODS

Collection of diseased sample and its preservation

A large number of diseased lentil plants was collected in the month of February 2007 from different lentil growing fields near Medininagar District, Palamu, Jharkhand (23° 52 N Lat. and 84° 17' E Long.) at Shahpur- Garhwa State Highway. The entire plant was uprooted keeping the root intact, while stalks were cut in several pieces (2.5 cm length) and wrapped in sterilized cellophane wrapping paper, labeled and preserved at a low temperature from 15-20°C to prevent saprophytic growth and for further use.

Isolation of the pathogen

Fusarium oxysporum f. sp. lentis was isolated from the root of the infected lentil plant and used during the period of study.

Single spore culture

In all the cases, pure culture of the entire organism was obtained by single spore isolation using dilution (10^{-5} dillution range) plate technique. Single spore stalk cultures were maintained on PDA medium and preserved at low temperature (8-12°C), throughout the investigation. The cultures were transferred on fresh PDA medium regularly at three month intervals.

Preparation of the different concentrations of IAA

The sucrose - nitrate liquid culture medium was used as basal medium. Eleven different concentrations starting from 0.1 to 10.0 ppm were tried. The results were recorded in two and four weeks incubation periods. The different strengths of IAA were added separately into 100 mL of basal medium in 250 mL Erlenmeyer flasks under aseptic conditions. The pH of the medium was adjusted to 6.0 and sterilized in autoclave at 15 psi for 15 min. This was done to prevent the active principles of IAA from being destroyed at higher temperature. In order to protect against the adverse effect of light, the flasks were wrapped with black paper throughout the period of investigation. For preparation of different concentrations of IAA, the substances were first dissolved in a drop of methyl alcohol after which they were slightly warmed. After the media were prepared, inoculums were added to each of the flasks which were then incubated at 26 ± 2°C for two and four weeks. For each of the trials, five replicates were prepared. In each case, control flasks with inoculums but without IAA were kept as check. After two and four weeks of incubation, reading was taken and presented in Tables 1, 2, 3 and 4 respectively.

Statistical analysis

This experiment was carried out using two incubation periods and eleven concentrations of the IAA. Variation of data on these two sources were calculated statistically using analysis of variance and test of significance was estimated by comparing F- ratio with the expected value of the variance ratio at the 0.5 and 0.1 percent probability level.

RESULTS AND DISCUSSION

There was continuous increase in mycelial dry weight as the concentration of IAA increased up to 1.0 ppm. Beyond this concentration (2.0 to 10.0 ppm), subsequent decrease was recorded in mycelial dry weight. At the concentration level of 10.0 ppm, the mean dry weight reached almost the amount of dry weight recorded in the control. In four weeks incubation, mycelial accumulation was recorded more than two weeks old incubation (Table 1 and Figure 1). The effect of different treatments was found highly significant. Effect of incubation period was also found to be highly significant. Effect of treatment between different concentrations was found significantly different from the control (1.0 and 6.0 ppm level of IAA) (Table 2). The maximum number of macroconidial sporulation was recorded in the 1.0 ppm concentration of IAA. Increase in concentration from 0.1 to 1.0 ppm population of macroconidia ascended gradually. Further increase was recorded in concentration from 1.0 to 10.0 ppm, however, the population of macroconidia descended but the population was recorded slightly more than the control even in 10.0 ppm level of IAA (Figure 2). The analysis of variance of the data revealed that the effect of treatment was highly significant. Effect of

Table 1. Effect of different concentrations of Indol 3- acetic acid (IAA) on the mycelial growth, population and size of macroconidia, microconidia and chlamydospores of *Fusarium oxysporum* f. sp. *lentis* (Incubation period: two and four weeks; initial pH: 6).

S/N	Conc. of IAA (ppm)	Incubation periods (weeks)	Final pH	Dry wt. of mycelium (mg)	No. of spores in millions/100 mL medium			Size of spores in µm		
					Macro conidia	Micro conidia	Chlamydo spores	Macro conidia	Micro conidia	Chlamydo spores
1	0.0	2	6.1	224.37	12.50	133.58	1.17	26.20	8.10	6.00
		4	6.0	338.42	14.06	147.64	2.34	26.20	8.30	6.00
2	0. 1	2	6.1	232.61	14.84	138.66	3.90	26.20	8.20	6.10
		4	6.0	347.18	15.23	148.82	5.47	26.20	8.30	6.20
3	0. 2	2	6.1	238.72	16.40	145.30	4.30	26.30	8.30	6.20
		4	6.0	351.20	17.97	154.29	6.25	26.30	8.40	6.30
4	0. 4	2	6.1	243.16	16.80	149.99	5.47	26.30	8.30	6.00
		4	6.0	371.33	18.79	155.85	7.42	26.40	8.40	6.00
5	0.6	2	6.1	251.11	19.92	151.94	6.25	26.50	8.40.	5.90
		4	6.0	382.41	21.87	158.58	8.20	26.80	8.70	5.90
6	0. 8	2	6.1	263.26	21.09	157.02	5.42	27.60	8.90	5.40
		4	6.0	393.16	23.83	163.27	6.25	27.90	9.10	5.60
7	1.0	2	6.1	281.38	22.65	159.36	2.34	28.90	9.10	4.80
		4	6.0	417.24	27.73	167.18	4.30	29.30	10.20	5.10
8	2.0	2	6.0	273.13	22.03	157.41	3.90	26.30	8.60	5.10
		4	5.9	407.37	26.56	162.10	4.69	26.70	8.70	5.40
9	4.0	2	6.0	259.48	21.09	151.94	5.47	25.60	8.40	5.40
		4	5.9	384.62	22.26	157.02	8.20	26.30	8.60	5.60
10	6.0	2	5.9	239.18	20.31	147.65	6.25	25.20	7.90	6.10
		4	5.8	358.43	22.05	151.16	9.24	26.10	8.10	6.20
11	8.0	2	5.9	231.35	17.19	142.96	7.42	25.10	7.60	6.20
		4	5.7	343.28	19.14	150.38	11.33	25.80	7.80	6.20
12	10.0	2	5.9	228.16	12.89	133.59	8.20	25.10	7.10	6.20
		4	5.7	331.89	16.01	142.57	12.11	25.40	7.40	6.20

Table 2. Analysis of variance of the effect of different concentrations of Indol 3- acetic acid (IAA) on the dry weight of mycelium and population of macroconidia of *Fusarium oxysporum* f. sp. *lentis* (Incubation period: two and four weeks).

S/N	Source of variation	DF	Dry weight of mycelium			Macroconidia		
			SS	MSS	F	SS	MSS	F
1	Between treatments	11	1298086.90	118007.90	3.34***	639.76	58.16	6.43***
2	Between periods	1	25388.40	25388.40	7.17***	8.03	8.03	0.88
3	Error	11	389249.63	35386.33		99.33	9.03	
			CD (5%) = 75.80			CD (5%) = 1.21		

Table 3. Analysis of variance of the effect of different concentrations of Indol 3- acetic acid (IAA) on the population of microconidia, and chlamydospores of *Fusarium oxysporum* f. sp. *lentis* (Incubation period: two and four weeks).

S/N	Source of variation	DF	Microconidia			Chlamydospores		
			SS	MSS	F	SS	MSS	F
1	Between treatments	11	232908.06	21173.46	23.49***	913.00	83.00	29.98***
2	Between periods	1	690.80	690.80	0.77	2.29	2.29	0.83
3	Error	11	9871.73	897.43		630.47	2.77	
			CD(5%) = 1.21			CD(5%) = 2.07		

Table 4. Analysis of variance of the effect of different concentrations of Indol 3- acetic acid (IAA) on the length of macroconidia, microconidia, and diameter of chlamydospores of *Fusarium oxysporum* f. sp. *lentis* (Incubation period: two and four weeks).

S/N	Source of variation	DF	Macroconidia			Microconidia			Chlamydospores		
			SS	MSS	F	SS	MSS	F	SS	MSS	F
1	Between treatments	11	196.57	17.87	0.76	100.65	9.15	8.08***	103.29	9.39	1.35
2	Between periods	1	19.99	19.99	0.86	1.02	1.02	0.90	4.06	4.06	0.59
3	Error	11	256.74	23.34		12.43	1.13		76.34	6.94	
			CD (5%) = 1.95			CD(5%) = 0.42			CD (5%) = 1.06		

DF: Degree of freedom; SS: Sum of square; MSS: Mean sum of square; F: Fisher's ratio; C.D.: Critical difference.

incubation period was found not to be significant. Among the treatment of different concentrations, the 1.0 ppm was found highly significant from the level of 0.1, 0.2, 2.0 and 10.0 ppm as well as the control (Table 2). The best sporulation in terms of the maximum number of microconidia was recorded in 1.0 ppm of IAA concentration. Increase in concentration of IAA from 0.1 to 1.0 ppm was recorded in the population. Further increase was recorded in concentration from 1.0 to 10.0 ppm and it was found that the number of spores gradually descended, though it was not steeper than that of the macroconidial population (Figure 3). The analysis of variance of the treatment was revealed to be highly significant. Effect of incubation period was not significant. Among the treatment, control was found significant over all the level of IAA concentrations. The 1.0 ppm of IAA concentration was also found to be significant over other levels of IAA concentrations (Table 3). Population of chlamydospores was found different from the effect over sporulation of macro- and micro- conidia rather it was found as irregular (Table 1). The population of chlamydospores was recorded poorly in control (1.17 and 2.34 millions/100 mL medium) and the population was found to increase from 0.1 to 0.6 ppm of IAA concentrations. The population of this spore was then found to decrease with increase in concentration from 0.8 to 1.0 ppm of IAA concentrations. Moreover, the population was found to further increase with increase in concentration from 1.0 to 10.0 ppm (Figure 4). The maximum sporulation (8.20 and 12.11 millions/100 mL medium) was recorded in 10.0 ppm of IAA while the minimum sporulation (2.34 and 4.30 millions/100 mL medium) was recorded in 1.0 ppm of IAA concentration. The analysis of variance of the treatment was found as highly significant. Among the treatment of different concentrations, the 0.1, 1.0 and 10.0 ppm was found as highly significant while 0.6 and 10.0 ppm was found to be significant. Other treatments were found not to be significant. Effect of incubation period was found not to be significant (Table 3). The length of macro- and micro-

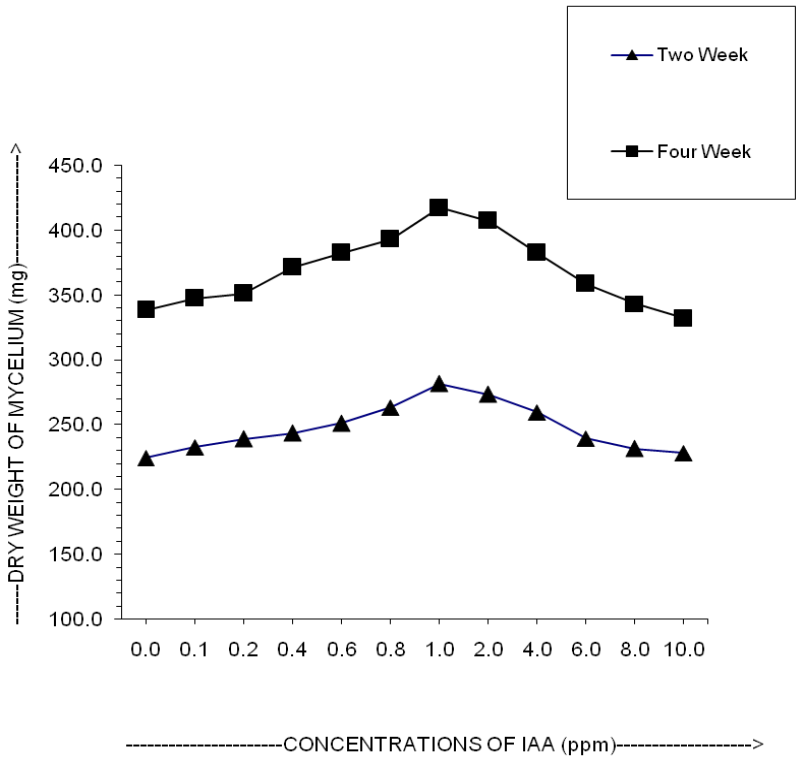


Figure 1. Effect of different concentrations of IAA on the dry weight of mycelium of *Fusarium oxysporum* f. sp. *lentis*.

Figure 2. Effect of different concentrations of IAA on the population of macroconidia of *Fusarium oxysporum* f. sp. *lentis*.

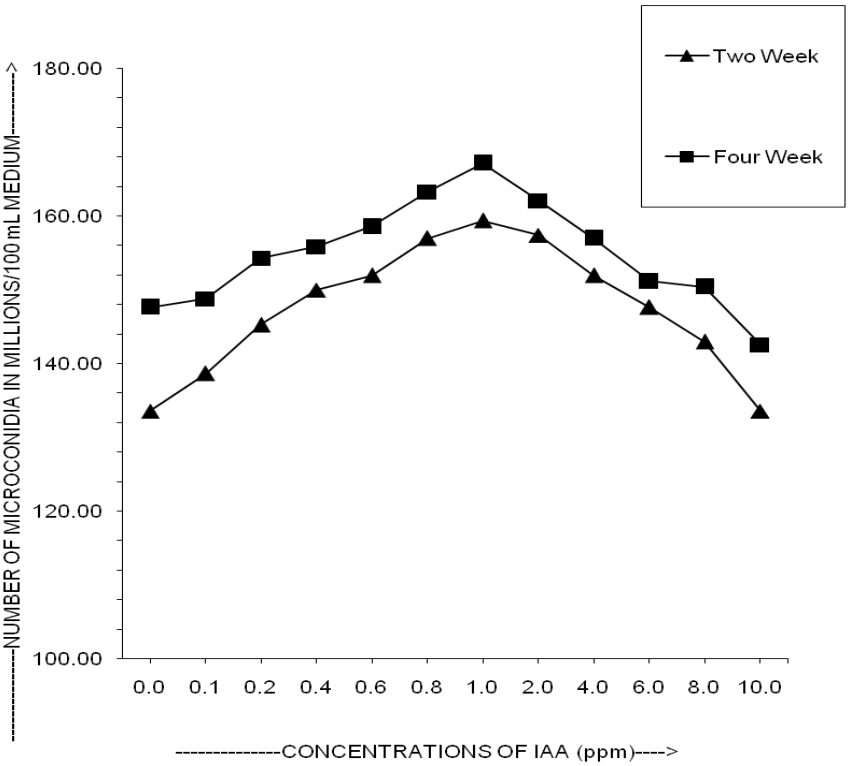


Figure 3. Effect of different concentrations of IAA on the population of microconidia of *Fusarium oxysporum* f. sp. *lentis*.

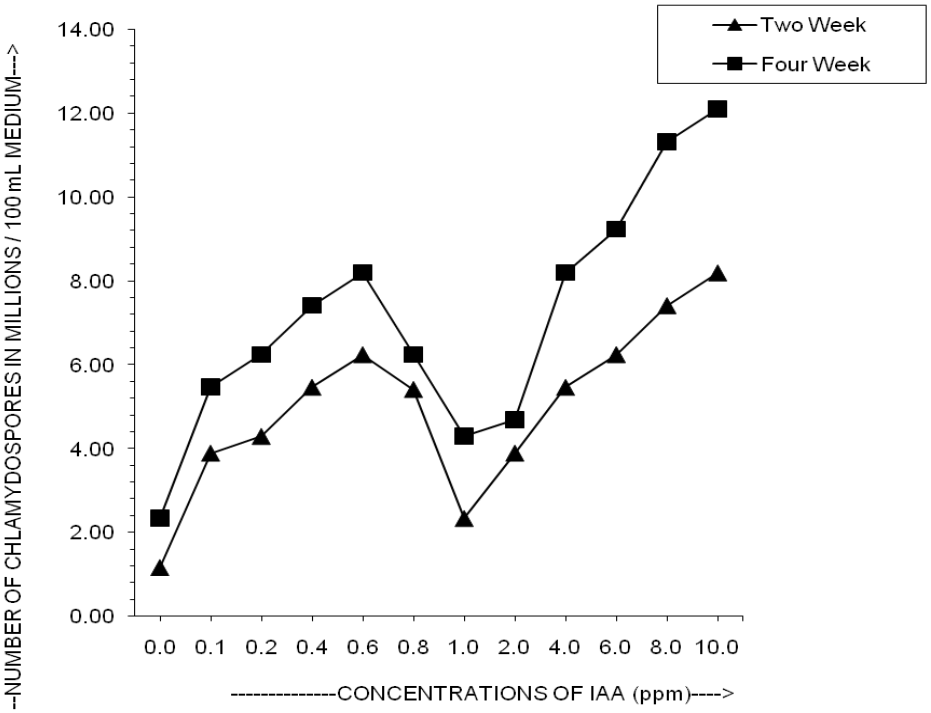
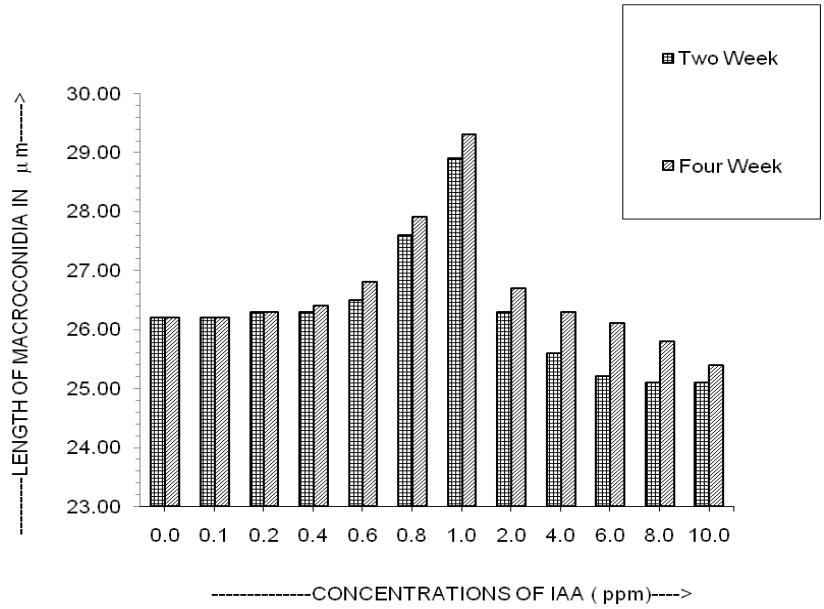


Figure 4. Effect of different concentrations of IAA on the population of chlamydospores of *Fusarium oxysporum* f. sp. *lentis*.



conidia was found to increase with increase in concentration of IAA from 0.1 to 1.0 ppm. Beyond the 1.0 pmm level of IAA concentration, the length of macro- and micro- conidia was found to decline. The maximum length of macroconidia (28.90 and 29.30 µm) and micro-conidia (9.10 and 10.20 µm) was recorded in 1.0 ppm level of IAA. Effect of incubation period on length was recorded minimum; however, it was more evident on the length of microconidia at 1.0 ppm level (Figures 5 and 6). In two weeks old culture, the largest diameter (6.20 µm) of

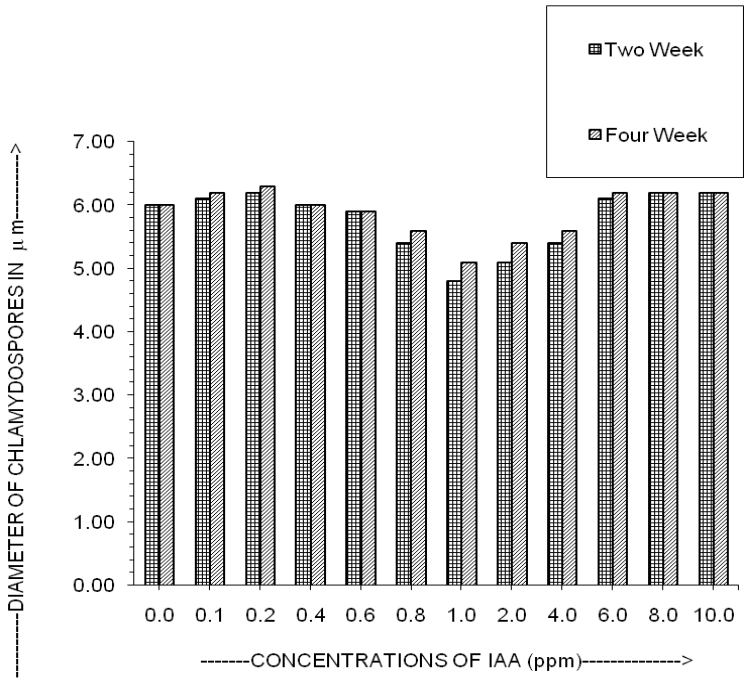


Figure 7. Effect of different concentrations of IAA on the diameter of chlasmydospores of *Fusarium oxysporum* f. sp. *lentis*.

chlamydospores was recorded in 0.2, 8.0 and 10.0 ppm of IAA level. On the other hand, the shortest diameter (4.80 µm) was recorded in 1.0 ppm of IAA level. In four weeks old culture, diameter of chlamydospores increased slightly in 0.1, 0.2, 0.8, 1.0, 2.0, and 4.0 ppm of IAA concentrations (Figure 7). In 0.4, 0.6, 8.0 and 10.0 ppm of IAA, there was no change in diameter recorded (Table 1). The analysis of variance of the treatment on length of macroconidia was found not to be significant. On the length of microconidia however, treatments were found as highly significant. Among the treatment, 1.0 ppm was found significant from other levels of IAA concentrations including control. Effect of incubation period was found not significant for the length of both macro- and micro-conidia. Effect of treatment and incubation period on diameter of chlamydospores was found not significant (Table 4).

The importance of auxins in plant growth and development is well known. The role of this substance in fungi however is yet to be elucidated. Some fungi and microorganisms, for example, to include bacteria are found to respond to plant growth hormones added into the culture media (Wolf, 1937; Gruen, 1959). Many papers published concerning the effect of IAA in fungi, particularly pathogenic fungi, are enumerated here:

Effect of growth accelerants have been studied on *Achyla bisexualis* and *Saprolegnia ferax* by Wolf (1937), on some species of *Phytophthora* by Mehrotra (1951), on *Phytophthora coctorum* by Schwinn (1964, 1965), on *Colletotrichum capsici* f. *cyamopsicola* by Agnihotri and Prasad (1965), on *Macrophomia phaseoli* (Maubl.) by Singh (1964), on *Pestalotia* theae and *Sphaerostilbe* by Roy (1964), on *Fusarium oxysporum* f. *cumini* by Sankhala et al. (1965), on *Fusarium vasinfectum* by Bouiillenne and Bouiillenne (1951), on *Fusarium moniliforme* Scheld. (*Gibberella fujikori)* (Sand) (Wr) by Adamiec (1966), and on *Fusarium oxysporum* f. *udum* by Prasad and Chaudhary (1976). Studies on the effect of IAA on the mycelial growth and sporulation of *Fusarium oysporum* f. sp. *lentis*, found that the lower concentration, 1.0 ppm of IAA levels was the best for the mycelial accumulation and sporulation of macroconidia. The microconidial sporulation also achieved the maximum number in lower concentration of IAA. Chlamydospores population was however exhibited as the best under higher as well as lower concentrations of IAA, but was exhibited as the least at 0.1 and 1.0 ppm of IAA. This behaviour of chlamydospores was also reported by Prasad and Chaudhary (1976) in *Fusarium oxysporum* f. sp. *udum* which is confirming the present work with regard to the concentration level for growth and sporulation of *Fusarium oxysporum* f. sp. *lentis.* Higher level of IAA (1.0 ppm) was reported as the best for mycelial growth and sporulation in *Macrophomina phaseoli* by Singh (1964). A very high concentration of IAA (100 ppm) was reported to act as the best for growth and sporulation in *Pestalotia theae* and *Sphaerostilbe repens* (Roy, 1964). In contrast to the above observation, inhibitory effect of IAA at higher concentration was reported by Schwinn (1964, 1965) for *Phytophthora cactorum*.

Inhibitory effect of IAA and GA_3 at higher concentration was also reported by Prasad and Chaudhary (1976) in *Fusarium moniliforme* versus *subglutinans* respectively. Sankhala et al. (1965) have also reported the inhibitory effect of IAA at higher concentrations in *Fusarium vasinfectum, F. oxysporum* f. *cumini* and *F. solani* respectively. Lower concentrations of IAA were found as the most suitable for sporulation of Chlamydospores in *Fusarium oxysporum* f. sp. *udum* (Prasad and Chaudhary, 1976). Similarly, higher concentrations also supported the maximum sporulation of chlamydospores in *Fusarium oxysporum* f. sp. *udum*.

Conclusions

Perusal of the result and the discussions indicates that inhibition of the growth and sporulation of the pathogen of the wilt disease of lentil was inhibited by the 1.0 ppm concentration of IAA. Growth of the mycelia which is indicated in the form of dry weight reduced up to the control. However spores population of macroconidia and microconidia reduced significantly in the presence of 1.0 ppm of IAA. Chlamydospore's population showed different trends. Its number reduced in the very lower concentration and very higher concentration of IAA. However, the number of macroconidia and microconidia reduced with increase in concentration from 0.01 ppm of IAA. This reduction in the number was observed in the 1.0 ppm concentration of IAA. Further increase in the concentration caused the population of both conidia to increase. On the other hand, the population of chlamydospores gradually decreased with increase in the concentration from 0.01 ppm of IAA to 1.0 ppm of IAA. However, the population of chlamydospores tends to increase with further increase in the concentration. Similar trends also appeared in the size of the spores and conidia.

The result of this study is in accordance with earlier observations that the effect of plant growth hormones on fungi depends on many factors, such as, kind of hormones, concentrations, and kind of fungi and their reproductive spores (Gruen, 1959; Mehrotra, 1951). According to the previous records, as well as results derived from the present work, it can be summarized that IAA play an important role of hormonal factor in *Fusarium oxysporum* f. sp. *lentis* for growth and sporulation, and inhibition of mycelia growth achieved completely. On the other hand, inhibition of sporulation was achieved but IAA failed to check completely. It can be concluded that IAA can be used as a potential bio-molecule for management of disease by altering IAA hormone concentration in the host.

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