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Effect of water soluble vitamins on growth, sporulation and chlamydospore formation of *Fusarium* spp.

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The effect of some vitamins on the growth, sporulation and chlamydospore formation on four *Fusarium* species (*viz. Fusarium equiseti*, *F. oxysporum, F. solani*. and *F. acuminatum)* were studied. The four species of *Fusarium* were isolated from wilt infected roots of pigeonpea plants. The addition of ascorbic acid exerted an inhibitory effect on the growth of *F. acuminatum* and *F. oxysporum*. Biotin accelerated the growth of *F. acuminatum* and had no effect on others. Nicotinic acid showed the inhibitory effect on the growth of *F. acuminatum* and *F. oxysporum*. It enhanced the growth of *F. solani* and *F. equiseti*. *F.acuminatum* and *F. oxysporum* obtained good growth on riboflavin wherease, riboflavin had no effect on others. In case of thiamine *F. acuminatum* and *F. oxysporum* attained good growth on this vitamin, whereas, it showed inhibitory effect on growth of *F. solani* and *F. equiseti*. The final pH of the medium drifted towards alkalinity, in all cases except it remained in acidic range in case of *F. oxysporum*. Addition of a mixture of vitamins, however, increased the dry weight of all four species of *Fusarium* except *Fusarium acuminatum*, in comparison to control. While all species showed excellent sporularion and poor chlamydospore formation.

Key words : Hydrogen-ion concentration, sporulation, clamydospore formation, *Fusarium* spp.

INTRODUCTION

Vitamins are organic substances and are needed in a minute quantity for the growth of an organism. According to Fries (1965) vitamins do not serve merely as a source of energy but they are necessary for the growth of different organism in a minute quantity. When used in case of fungi they function as part of coenzyme. Like other organisms, fungi also require minute amount of vitamins for their growth. A vitamin deficient fungus can be completely incapable of synthesizing a certain growth factor, or the biosynthetic capacity may be reduced which may be gained by an external supply of a favourable vitamin in the substrate.

Fungi which are unable to synthesize the vitamins are called vitamin deficient. Schopfer (1943) called them hetrotrophic fungi with respect to vitamins. A number of fungi are known to be vitamin-deficient but the needs of the various species differ and may exhibit partial or total as well as single or multiple deficiency. On the other hand fungi may or may not be dependent partially or totally on an exogenous supply of vitamins. Many of them including species of *Fusarium, Aspergillus* and *Penicillium* are able to grow on vitamin free substance and the addition of the vitamin may not significantly affect their growth. Such forms which do not need external supply of vitamins, are known as self-sufficient. As a rule, fungi have no requirement for fat –soluble vitamins, (vitamin A, D, E and K). Only water soluble vitamins are required by fungi (Dube, 1997).

Generally, an exogenous supply of vitamin accelerates the growth of fungi, though some of the organisms are reported to attain the same mycelial growth even on a vitamin free medium. Usually the addition of vitamins especially in high concentration exerts a harmful or inhibitory effect on the growth of the fungi. In the present investigation an attempt has been made to study the effect of some vitamins on the growth, sporulation and chlamydospore formation of four *Fusarium* species.

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MATERIALS AND METHODS

Four *Fusarium* spp. *viz*. *Fusarium equiseti*, *F. oxysporum, F. solani*. and *F. acuminatum* were isolated from wilt infected roots of pigeonpea plants which were collected from different places of Allahabad district and culture was maintained. There were eight treatments comprised of six treatments contained only one vitamin at a time and one treatment had all six vitamins and one treatment for control (without any vitamin). All treatments were replicated thrice. The PDA medium was used for inoculation of *Fusarium* spp. Vitamins were added in the basal medium at the following concentrations: Ascorbic acid 50 µg, Biotin 10 µg, Folic acid 20 µg, Nicotinic acid 50 µg, Riboflavin 50 µg and Thiamin 100 µgl. Inoculations were made in Petridishes from pure culture and incubated at 27°C for seven days. The mycelium was harvested by centrifugation for 20 min at 6000 g and dry mycelial weight was obtained by filtering the sample cultures through a pre-weighed filter paper (Whatman no.42) and drying in an oven for 24 hrs. at 50°C (Wang and Rakshit, 1999).

RESULTS AND DISCUSSION

Addition of a mixture of vitamins, however, increased the dry weight of all four species of *Fusarium* except *Fusarium acuminatum*, in comparison to control. While all species showed excellent sporularion and poor chlamydospore formation. The pH of the medium in all cases drifted towards alkalinity except *F. oxysporum*. Similar results were obtained by Lilly and Barnett (1951) for *Sphaeropsis malorum* and *Phoma betae*. Ellen and Walsh (1980) found that a vitamin mixture provided a partial replacement of potato extract but no other group of compounds had an effect when used alone for the growth of *F. moniliforme* and other fungi.

None of the *Fusarium* species needed ascorbic acid in general. The addition of ascorbic acid exerted an inhibitory effect on the growth of *F. acuminatum* and *F. oxysporum* as it could induce poor growth for these organisms.

In the present investigation biotin accelerated the growth of *F. acuminatum*. Similar results were also obtained by Chattopadhyay and Nandi (1981) who found that the *Fusarium moniliforme* var. *subglutinans* was favoured by biotin and only slightly by thiamin in the medium. However, this vitamin had no effect on *F. oxysporum* and *F. solani* and *F. equiseti*. The final pH of the medium moved towards alkalinity in all cases. Meyer and Maraite (1967) observed that a medium supplemented with biotin will support growth *F. oxysporum* f. sp. *melonis*. (Table 1).

Table 1 : Effect of vitamins on dry weight, sporulation, change in pH and chlamydospore formation of *Fusarium acuminatum*

Vitamins	Dry wt. (mg)	Sporulation	Chlamydospore formation	Final pH
Ascorbic acid	68.0	Excellent	Poor	7.2
Biotin	84.1	Excellent	--	7.2
Folic acid	75.0	Excellent	--	7.5
Nicotinic acid	68.8	Excellent	Poor	7.0
Riboflavin	85.9	Excellent	--	7.5
Thiamin	92.9	Excellent	--	7.8
All the six vitamins	76.7	Excellent	--	7.2
Control	84.2	Excellent	Poor	7.4

CD= 2.49 at 5%level of significant

Table 2 : Effect of vitamins on dry weight, sporulation, change in pH and chlamydospore formation of *Fusarium oxysporum*.

Vitamins	Dry wt.(mg)	Sporulation	Chlamydospore formation	Final pH
Ascorbic acid	28.1	Good	Poor	7.0
Biotin	86.7	Good	Poor	7.5
Folic acid	48.3	Good	--	7.2
Nicotinic acid	29.8	Good	Poor	7.0
Riboflavin	55.2	Good	--	7.2
Thiamin	58.0	Excellent	--	6.8
All the six vitamins	76.7	Excellent	Poor	7.2
Control	40.0	Good	--	7.2

CD= 2.29 at 5%level of significant

Table 3 : Effect of vitamins on dry weight, sporulation, change in pH and chlamydospore formation of *Fusarium solani*

Vitamins	Dry wt. (mg)	Sporulation	Chlamydospore formation	Final pH
Ascorbic acid	52 .0	Excellent	Poor	7 .1
Biotin	64 .8	Good	--	7 .5
Folic acid	67 .8	Excellent	--	7 .5
Nicotinic acid	75 .7	Good	--	7 .5
Riboflavin	67 .9	Good	--	7 .5
Thiamin	47 .3	Good	Poor	7 .5
All the six vitamins	69 .3	Excellent	--	7 .5
Control	60 .0	Excellent	Poor	7 .2

CD= 1.1 at 5%level of significant

Table 4 : Effect of vitamins on dry weight, sporulation, change in pH and chlamydospore formation of *Fusarium equiseti*

Vitamins	Dry wt. (mg)	Sporulation	Chlamydospore formation	Final pH
Ascorbic acid	50.0	Good	--	7.0
Biotin	65.3	Good	--	7.5
Folic acid	68.9	Excellent	--	7.5
Nicotinic acid	78.7	Excellent	Poor	7.5
Riboflavin	68.7	Excellent	--	7.5
Thiamin	43.5	Good	--	7.5
All the six vitamins	77.5	Excellent	--	7.3
Control	68.0	Excellent	Poor	7.0

CD= 2.18 at 5%level of significant

The dry weight results showed that folic acid had no marked effect on the growth of present organisms as their dry weight were statistically similar to that produced on medium devoid of vitamins. The pH of the medium in all cases drifted towards alkalinity. (Table 2).

Nicolinic acid showed the inhibitory effect on the growth of *F. acuminatum* and *F. oxysporum*. It enhanced the growth of *F. solani* and *F. equiseti*. The final pH of the medium used by *F. acuminatum*, *F. oxysporum, F. solani* and *F. equiseti* were 7.0, 6.8, 7.5 and 7.5 respectively. (Table 3).

F. acuminatum and *F. oxysporum* obtained good growth on riboflavin whereas, it had no marked effect on the growth of *F. solani* and *F. equiseti.* The final pH of the medium drifted towards alkalinity in all cases.

F. acuminatum and *F. oxysporum* attained good growth on thiamin, whereas, it showed inhibitory effect on growth of *F. solani* and *F. equiseti*. The final pH of the medium used by *F. acuminatum*, *F. solani* and *F. equiseti* drifted towards alkalinity, whereas, it remained in acidic range in case of *F. oxysporum*. (Table 4). Though external supply of individual vitamins had affected growth of the present species of *Fusarium* yet no marked difference in sporulation was observed. Sporulation of all the four species of *Fusarium* in general varied from excellent to good in media added with an individual or mixture of vitamins or devoid of vitamins. It can be concluded from the present study that all the four *Fusarium* species were capable of synthesizing the essential vitamins needed for their growth as all of them grew moderately on vitamin free medium. Out of four species of *F. acuminatum, F. oxysporum, F. solani,* and *F. equiseti* attained similar growth on a mixture of vitamins and control (no vitamin added). However, the growth of *F. acuminatum* was enhanced in the vitamin mixture. Hussein (1957) found that the *Rhizoctonia* and *Fusarium* showed partial deficiencies in thiamin, biotin, and inositol, both grown on vitamin-free medium. El-Abyad and Ramadan (1979) reported that the fungus *F. oxysporum* f. sp. *vesinfectum* responded favourably to all of the utilized vitamins in almost all the concentrations where germination, growth, and sporulation were substantially greater than the controls. Among the vitamins used, the fungus appeared to be highly sensitive to thiamin and pyridoxine, moderately sensitive to inositol and pantothenate, and least affected by folic acid. Prasad (1972) found that on the basis of sporulation and fungal mat production both *F. oxysporum* and *F. moniliforme* v.*subglutinans* were auxoheterotrophic for thiamin, biotin, inositol, riboflavin and pyridoxine. Hulya and Tarhan (2006) observed that the superoxide dismutase activities in *F. equiseti*, and *F. acuminatum* strains decreased significantly with the increases in Vitamins C, B_1 and B_6 concentrations.

This clearly shows that the above fungus could synthesize the vitamins necessary for its growth but addition of vitamins accelerated the growth. The results from the above study show that individual exogenous supply of a vitamin either do not influence the growth of the present organisms, or has accelerated it or exerted an inhibitory effect.

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