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EFFECT OF SOIL MOISTURE AND TEMPERATURE ON THE POPULATION OF *VERTICILLIUM CHLAMYDOSPORIUM* GODDARD

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Abstract

Population of *Verticillium chlamydosporium* was examined at different soil moisture and temperature regimes. A significant ($p<0.001$) reduction in population of *V. chlamydosporium* was observed at 0 & 100% MHC after 4 weeks. Whereas 50 & 75% moisture enhanced the germination of chlamydosporium and an increase in population of *V. chlamydosporium* was observed. A moderate effect on survival of chlamydospores was observed at 25% MHC. In another experiment effect of soil temperature on population of *V. chlamydosporium* in moist and dry soil was observed. Population of fungal chlamydospores was declined at 30, 40 & 50°C in wet and dry soils. A moderate temperature (20°C) significantly ($p<0.001$) increased the fungal population in wet soil whereas fungal survival decreased slightly at 10°C in both wet and dry soils.

Introduction

Verticillium chlamydosporium Goddard, is a facultative parasite of eggs and females of cyst and root knot nematodes (Kerry, 1975; Godoy *et al.*, 1983; Freire & Bridge, 1985; Zaki & Maqbool, 1993), The fungus can survive in soil and on the rhizosphere of plants as well as destroy nematodes when it contacts them. *V. chlamydosporium* is one of the fungi involved in the natural control of cereal cyst nematodes in cereal monoculture in northern Europe, and is important in the suppression of several other cyst nematode pests (Kerry, 1988). There is a considerable variation within the species of *V. chlamydosporium* regarding temperature, optimum growth rate on agar, ability to produce chlamydospores, capacity to colonize the rhizosphere of plants and ability to infect nematodes eggs (Kerry *et al.*, 1986; Irving & Kerry, 1986; de Leij & Kerry, 1991). Chlamydospores and some hyphal fragments of *V. chlamydosporium* (isolate 10) rapidly proliferated and survival upto 110 days in non-sterile soil (de Leij, 1992). In the present study, we aimed to examine the survival of *V. chlamydosporium* in soil i) under different moisture levels at 25±8°C, and ii) Continuos temperature cycle in wet and dry soil.

Materials and Methods

Verticillium chlamydosporium Goddard (isolate Vc 10) selected for present study was originally isolated from eggs of root knot nematode *Meloidogyne incognita*, provided by Dr. B. R. Kerry of Rothamsted Experimental Station, UK. Three weeks old chlamydospores grown on cornmeal sand medium (w/w) were collected on 10 µm sieve. Chlamydospores were mixed in approximately 1:10 ratio (chlamydospores: sand , w/w) and mixed to sandy loam, pH 8.1 @ 1% ($3\text{-}6\times10^5$ chlamydospores g^{-1} soil).

Soil samples taken from each replicate were air dried for 24 hrs at room temperature and then 0.2 ml of soil dilution series (10^2 & 10^3) was spread over semi selective media. Each dilution Petri plate was replicated twice. Colonies emerging from viable chlamydospores were observed under microscope after 10-12 days incubation on 25±2°C. Number of viable cfu was calculated per g soil.

Effect of different soil moisture levels: Sand meal inoculum of fungal chlamydospores was incorporated in 100 g fine sieved soil @ 1%, 20 g of soil was transferred in 6.5 cm diam. Petri plates. Different moisture levels (i.e. 25, 50, 75 and 100%) were adjusted in Petri plates according to treatment plan. Each treatment was replicated thrice. Set of plates receiving no water (0% moisture) served as control. Plates were randomized and placed in incubator at 28±2°C. One-g soil sub sample from each replicate was taken at the interval of 1, 3, 5, 7, 14, 21 & 28 days and population g^{-1} soil was determined.

Effect of Different soil Temperatures: Petri plates containing fungal incorporated soil (fungal inoculum @1%), 20 g in each were kept dry or moisture (50% WHC) and was replicated thrice. Plates were randomized in plastic trays and placed in incubators at different temperatures (i.e. 10, 20, 30, 40 & 50°C). A sub sample (1 g soil/replicate) was taken at 0, 1, 3 & 7 days of interval.

Results

Effect of different soil moisture levels: Fungal chlamydospore incorporated in cultivated sandy loam soil exhibited variable response to different moisture levels (i.e. 0, 25, 50, 75 & 100 %). Maximum suppression in fungal population was observed in two moisture extremes i.e. 0 & 100%. Greater reduction in population of *V. chlamydosporium* was observed after 21 and 28 days of interval, in treatments where soil was maintained at 0% ($3x10^3$ cfu g^{-1} & $6x10^3$ cfu g^{-1} soil respectively) and 100% moisture ($2x10^3$ cfu g^{-1} & $4x10^3$ cfu g^{-1} soil respectively) compared to other treatments. Maximum number of viable chlamydospores ($29x10^3$ cfu g^{-1} soil) was obtained in 75% moisture after 28 days of interval, followed by 50% moisture level ($24x10^3$ cfu g^{-1} soil) compared to other treatments (Fig 1).

Effect of soil temperatures: After 7 days exposure at 40 & 50°C in wet soil fungal propagules were eliminated, whereas in dry soil population reduced by 76% and 84% at 40 & 50°C exposure respectively. No considerable reduction in fungal population was observed in wet and dry soil at 10°C & 20°C. At 30°C temperature both in wet and dry soil, moderate suppression in population was observed compared to lower and higher temperature ranges (Fig 2).

Discussion

In soil environment abiotic factors like temperature and moisture play an important role in the establishment of an organism. Temperature and moisture has a major effect on cellular activities of fungus. The results indicate that chlamydospores of *V. chlamydosporium* exposed to lower (0%) and higher moisture levels (100%) had lost their viability after 4 weeks. In wet soil at 40 and 50°C fungal propagules were eliminated completely. Suppression in viability of fungal

chlamydospores may attribute to sublethal heating which caused cracks in the rind, increased leakage to the partial or complete inactivation, as reported by Lifshitz *et al* (1983) in case of sclerotia of *S. rolfsii.* According to Smith (1972) dried and re-moisture sclerotia of *Sclerotium rolfsii* leaked large quantities of sugars and amino acids and subsequently rotted in soil. Inactivation of chlamydospores of *V. chlamydosporium* depends on time-temperature exposure and environment into which propagules are introduced. In the present study, moisture and temperature at different exposure of time induced considerable effects on chlamydospores. The protein contents of persisting fungal structure like chlamydospores are more readily denatured by moist than by dry heat, which make impossible to rejuvenation (Crisan, 1973). The evidence mentioned here support our results.

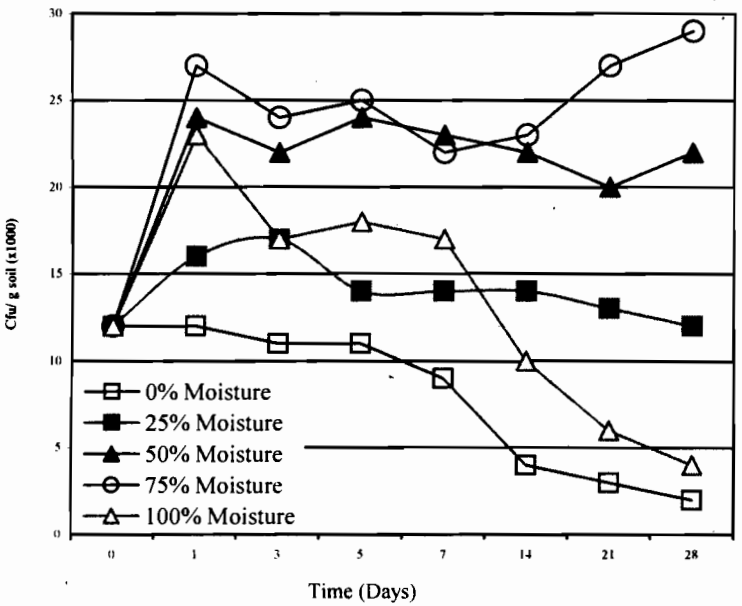

Fig 1. Effect of different moisture levels on the survival of *Verticillium chlamydosporium* in soil.

Signifincant level (p)
Source: Time=15.42(***), Moisture=279.23 (***), TimexMoisture=29.72(***),***, 0.001

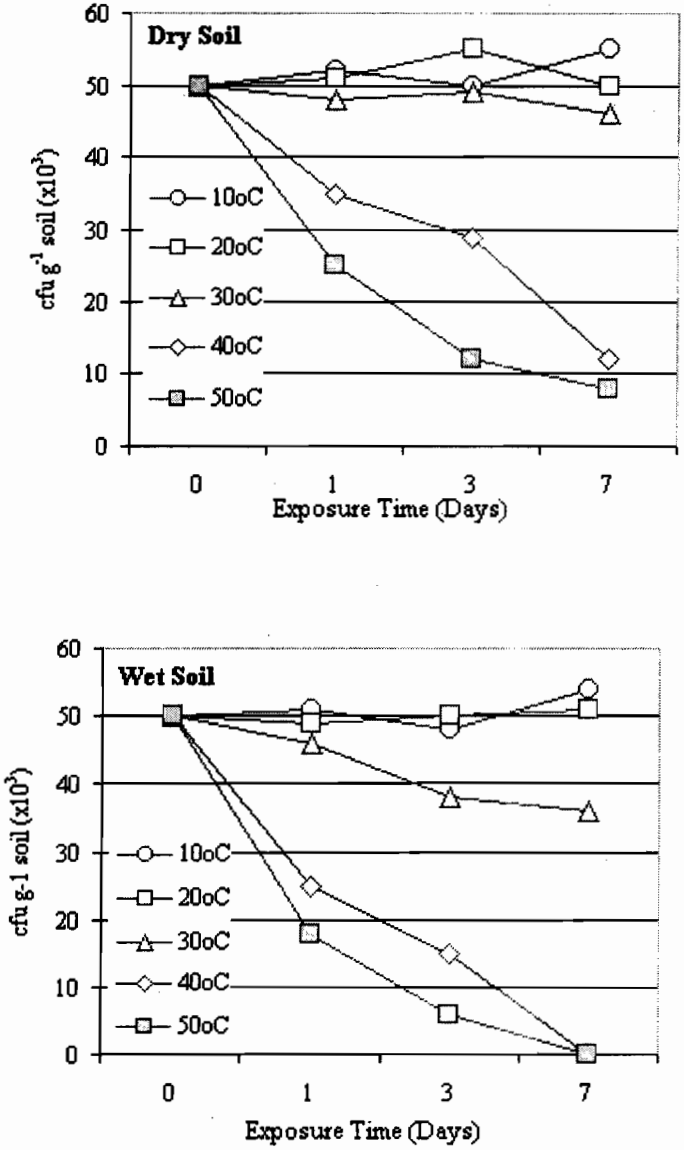

Fig 2. Effect of continuous temperature on viability of chlamydospores of *Verticillium chlamydosporium* Goddard, in wet and dry soil *in vitro*.

Source
Temperature= 954.61 (***), Time= 395.27 (***), Treatments= 124.17 (***), Temp. x Time= 136.1 (***), Temp.x Treatt.= 14.16 (***), Temp.xTimexTreatt.= 2.89 (**)
, 0.01; *, 0.001

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