BIOCONTROL OF *HETERODERA AVENAE* INFECTING WHEAT USING DIFFERENT INOCULUM SEQUENCE OF *VERTICILLIUM CHLAMYDOSPORIUM*

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(Accepted July, 1997)

Ecofriendly management of *Heterodera avenae* using fungus *Verticillium chlamydosporium* isolated from the local fields was carried out under pot trials. Fungus was multiplied on cheaper wheat bran substrate. Eight gram of such fungal containing wheat bran were inoculated in three different sequences viz. (i) Fifteen days prior to nematode inoculation. (ii) Simultaneously with nematode and (iii) Fifteen days after nematode inoculation in 15 cm diameter earthen pots containing 1Kg of sterilized soil. Spore load per gram was estimated using standard formula. Observations revealed that the timing of the fungal treatment greatly affected the plant growth characters. The reduction in the plant growth characters were more discernible when the nematode inoculation was given prior to the fungal inoculation. However, better plant growth and appreciable reduction in cyst formation per plant was noted in plants where fungal inoculation preceded nematode inoculation. Young females, cysts, and eggs were found colonized by the fungal hyphae. Prior application of biocontrol agent proved beneficial in managing the nematode population to an appreciable level.

**Key Words**: Biocontrol, *Heterodera avenae*, Wheat *Verticillium chlamydosporium*.

Various physical and chemical agents can be used to keep the pests below damaging level to the host plant. The problems in the use of chemical pesticides have enhanced the development of biocontrol methods for controlling nematodes by various antagonist organisms. There has recently been an awareness of using nematophagous fungi naturally occurring in soil for the effective control of plant parasitic nematodes. Fungi are effective in competition and hyperparasitism, easier to handle, non-hazardous and their use considerably reduces the total requirement of nematicide for effective nematode management (Trivedi, 1992). *Verticillium chlamydosporium* attributes qualities of a successful biocontrol agent against nematodes. Kerry (1975) found *Verticillium chlamydosporium* as the most important egg parasite capable of infecting all developmental stages of *Heterodera avenae*. Although *Verticillium chlamydosporium* can destroy the eggs, larvae, and cysts of *Heterodera avenae*, its best exploration as a biocontrol agent depends upon the way of its application against nematode. An attempt has therefore, been made in this investigation to study the right time of inoculation of nematophagous fungus *Verticillium chlamydosporium* for the maximum control of *Heterodera avenae*.

**MATERIALS AND METHODS**

In the present experiment 8g of *Verticillium chlamydosporium* multiplied on wheat bran was used to inoculate 1Kg of sterilized soil filled in 15cm diameter earthen pots in three different inoculation sequences viz.: (i) 15 days prior to nematode inoculation. (ii) Simultaneously with nematode. (iii) 15 days after nematode inoculation

Uninoculated plants served as control. Nematode alone treated plants were kept as check.

The fungus culture was prepared by inoculating moist steam sterilised wheat bran with 7 days old culture of *Verticillium chlamydosporium* obtained on PDA. The flasks were incubated at 29°C for 15 days. The spore load was counted by washing 1g fungus colonized substrate through sieves of 50 and 10 mm aperture so as to remove the culture media, conidia, and small hyphal fragments leaving mainly chlamydospores. Spores left were counted with the help of haemocytometer. Spore load per gram (SPLG) was estimated by using following formula (Mathur and Bennum, 1974).

\[
SPLG = \frac{N \times V \times 1000}{W}
\]

N = Numbers of spores in central squares of haemocytometer.
V = Volume of mounting fluid added to the substrate.
W = Weight of the substrate.

Seeds were sown in the pots and thinning was done to one plant per pot after germination. Nematode
inoculation was done by pipetting, 1000 freshly hatched juveniles suspended in sterile distilled water into the three holes equidistant around the seedlings. After inoculation holes were plugged with autoclaved soil. Each treatment was replicated 4 times in a complete randomised design. Plants were uprooted after 90 days and readings on plant growth characters such as length, fresh weight and dry weight of root, shoot and ear were noted. Observations were also made on number of cysts per plant and number of eggs per cyst. For counting number of eggs per cyst, the cyst were stained with 0.1% acid fuchsin and were observed under stereoscopic binocular microscope. Both attached and detached cysts were also observed under microscope after staining them with 0.1% cotton blue for the presence of nematophagous fungus. Lactophenol was used as a mounting medium. All the data were analysed statistically.

**OBSERVATIONS**

Data in the present investigation revealed that the timing of the fungal treatment greatly affected the plant growth characters.

A comparative assessment of the nematode penetration and damage caused by *Heterodera avenae* was noticed that the reduction in the plant growth characters viz. root-shoot length and weight were more discernible when the nematode inoculation was given prior to the fungal inoculation. The plants showed stunted growth, reduced vigour and yellowing of leaves. Great damage was inflicted to the ear also by considerable reduction in its length, fresh and dry weight. The roots when studied were found crowded with white glistening cysts having high cyst contents (eggs and larvae) and only few hyphae and spores of *Verticillium chlamydosporium* present, showing higher penetration and damage of the wheat roots by the nematode (Table-1).

However, maximum reduction in the root-shoot length, fresh and dry weights were observed in the plants treated with nematode alone. The plants were noticeably stunted with smaller leaves and petioles. Most of them showed growth, with only few yellowish green leaves appeared functional at the end of the experiment. The cysts per plant were present to their maximum limit with highest number of eggs and larva in them.

Better plant growth in terms of root-shoot and ear length were envisioned in the treatment where fungal inoculation preceded nematode inoculation. Appreciable reduction was observed in the cyst length, fresh weight and dry weight of root, shoot and ear were noted. Observations were also made on number of cysts per plant and number of eggs per cyst. For counting number of eggs per cyst, the cyst were stained with 0.1% acid fuchsin and were observed under stereoscopic binocular microscope. Both attached and detached cysts were also observed under microscope after staining them with 0.1% cotton blue for the presence of nematophagous fungus. Lactophenol was used as a mounting medium. All the data were analysed statistically.

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**Table 1: Effect of different inoculum sequences of *Verticillium chlamydosporium* for controlling *Heterodera avenae* on wheat (Mean of four replicates).**

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Length (cm)</th>
<th>Fresh wt. (g)</th>
<th>Dry wt. (g)</th>
<th>Ear length (cm)</th>
<th>Ear wt. (g)</th>
<th>Total no. of cysts/plants</th>
<th>Total no. of eggs/cyst</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Shoot Root</td>
<td>Shoot Root</td>
<td>Shoot Root</td>
<td>Shoot Root</td>
<td>Shoot Root</td>
<td></td>
<td></td>
</tr>
<tr>
<td>i</td>
<td>2 3 4 5</td>
<td>6 7 8 9 10</td>
<td>11 12</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-V-WB(at a time)</td>
<td>69.75 31.97</td>
<td>19.65 6.12</td>
<td>2.06 0.67</td>
<td>15.67 3.86</td>
<td>0.76</td>
<td>33.5 129</td>
<td>(5.86) (11.38)</td>
</tr>
<tr>
<td>N(p)+V+WB</td>
<td>67.7 29.97</td>
<td>18.82 5.7</td>
<td>1.82 0.6</td>
<td>15.37 3.73</td>
<td>0.58</td>
<td>59.75 188.72</td>
<td>(7.78) (13.74)</td>
</tr>
<tr>
<td>BV-WB(p)+N</td>
<td>84.12 33.2</td>
<td>20.8 6.62</td>
<td>2.23 0.7</td>
<td>16.07 4.87</td>
<td>0.88</td>
<td>9.75 94.5</td>
<td>(3.26) (9.75)</td>
</tr>
<tr>
<td>N-WB(at a time)</td>
<td>59.95 27.05</td>
<td>14.82 3.42</td>
<td>1.5 0.44</td>
<td>10.05 1.97</td>
<td>0.197</td>
<td>69 296</td>
<td>(8.36) (17.23)</td>
</tr>
<tr>
<td>N(p)+WB</td>
<td>60.55 26.8</td>
<td>14.47 3.07</td>
<td>1.5 0.39</td>
<td>9.95 0.90</td>
<td>0.140</td>
<td>73 293</td>
<td>(8.6) (17.12)</td>
</tr>
<tr>
<td>N+W(B)p</td>
<td>62.27 28.77</td>
<td>15.85 4.2</td>
<td>1.65 0.52</td>
<td>11 1.15</td>
<td>0.278</td>
<td>61 284.5</td>
<td>(7.86) (16.9)</td>
</tr>
<tr>
<td>N alone</td>
<td>56.37 26.0</td>
<td>14.12 2.97</td>
<td>1.4 0.33</td>
<td>9.77 0.85</td>
<td>0.122</td>
<td>71 300</td>
<td>(8.45) (17.38)</td>
</tr>
<tr>
<td>Control</td>
<td>76.80 33.15</td>
<td>20.72 6.6</td>
<td>2.14 0.69</td>
<td>17.05 5.17</td>
<td>0.96</td>
<td>0 0</td>
<td>(1) (1)</td>
</tr>
<tr>
<td>SEMs</td>
<td>0.68 0.21</td>
<td>0.17 0.18</td>
<td>0.04 0.02</td>
<td>0.19 0.18</td>
<td>0.3</td>
<td>0.2 0.15</td>
<td></td>
</tr>
<tr>
<td>CD at 5%</td>
<td>1.39 0.45</td>
<td>0.36 0.37</td>
<td>0.07 0.03</td>
<td>0.39 0.37</td>
<td>0.62</td>
<td>0.43 0.32</td>
<td></td>
</tr>
<tr>
<td>CD at 1%</td>
<td>1.86 0.59</td>
<td>0.48 0.5</td>
<td>0.10 0.05</td>
<td>0.52 0.50</td>
<td>0.84</td>
<td>0.58 0.43</td>
<td></td>
</tr>
</tbody>
</table>

N=Nematode, WB=Wheat bran, V-Verticillium *chlamydosporium*. (p)=prior
Figures in parenthesis are √n+1 transformed values
Biocontrol of *Heterodera avenae* infecting wheat production per plant and the number of egg per cyst. The size of the cysts were also greatly reduced in this treatment. Young females and cysts were bearing hyphae of *Verticillium chlamydosporium*. Mature cysts when crushed showed colonisation of the eggs of *Heterodera avenae* by the fungal hyphae.

Simultaneous inoculation of nematode and fungus showed results in between. There moderate increase in plant growth characters when compared with only nematode treated plants. Though the number of cysts per plant decreased thereby reducing the disease incidence but the effect was not so pronounced when compared with plants where fungus was introduced prior to nematode.

In terms of plant growth characters wheat bran alone treated plant attributes the most followed by uninoculated control.

All the parameters were found statistically significant among the treatments.

**DISCUSSION**

*Verticillium chlamydosporium* is capable of infecting all developmental stages of *Heterodera avenae* including eggs, larvae, young females and cysts (Kerry, 1975; Morgan Jones *et al.*, 1983). This fact was exploited for effective control of the cereal cyst nematode (*H. avenae*) and to find out the most effective sequence of nematode inoculation. The protective effect of the three sequences were measured and the best protection against *Heterodera avenae* on wheat was recorded in all pots with the application of fungus *Verticillium chlamydosporium* 15 days prior to the nematode inoculation. This cab be attributed to the fact that the fungus occupied the niche before nematode attack, making the environment unfavourable for the nematode development. Cabanillas and Barker (1989) obtained two fold increase in tomato yield and 56% suppression of galling with two applications of *Paecilomyces hilaracinus* to days before transplanting and at transplanting against *Meloidogyne incognita*. O'Hara and Jatala (1985) and Jatala (1986) recorded destruction of embryo of *Meloidogyne incognita* by *Paecilomyces hilaracinus* within 5 days of inoculation.

Wheat bran used in the experiment provided a good substrate for the multiplication of the fungus *Verticillium chlamydosporium* mainly because it is economical, easily available and also the spore load is very high. Thus this cheaper substrate can be exploited by the farmers to protect their crop from the severe attack of *Heterodera avenae*. Sharma & Trivedi (1987) found it best substrate for culturing *Paecilomyces hilaracinus*. The physical nature of the soil viz. improved tilth, aeration and abundance of nutrients were also changed by the pretreatment of the soil with the substrate and the metabolites released as a result of its decomposition made the environment highly unsuitable for the disease development. Decomposition products of wheat bran like aldehyde, isobutylaldehyde, isovaleraldehyde, I-methyl butanol and ethanol have been found to stimulate fungal activity (Linderman and Gilbert, 1969; Owens *et al.*, 1969). The fungus colonized on wheat bran attacked the eggs of *Heterodera avenae* thus reducing the inoculum level (eggs and juveniles). Pandey & Trivedi (1992) explained its mode of action on *Meloidogyne sp*. They suggested that the fungus penetrated the eggs, colonised them and prevented hatching. Further it disrupted the cuticle and egg shell and the hyphae readily proliferated endogenously. The invaded larvae became totally necrotic and disintegrated. Addition of substrate and fungus may also failed the root to exude attraction factors necessary for proper orientation of nematode juveniles (Bird, 1959, 1960). Thus a highly unfavourable environment was created by the pretreatment of soil with the fungal colonised sunstrate.

When the nematode inoculation preceded fungal treatment the nematode increase and the damage has already been occurred before the fungal application and therefore, the fungus could not help much in controlling the disease severity. This was also reflected by the number of cysts developed per plant and also by the number of eggs counted per cyst which were significantly more in this treatment. The plants in these pots showed retarded growth, poor plant development and chlorosis of leaves.

Thus the present study reveals that the pretreatment of plant with fungus proved beneficial in controlling the nema population already present in the soil as well as in reducing the further multiplication of the pathogen.

Authors are grateful to the Head, Botany Department for providing the facilities and D.S.T., New Delhi for financial assistance.
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