Application of *Dactylaria brochopaga* for The Control of Root-gall Disease of Balsam (*Impatiens balsamina*)

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**Abstract**  
Experiments were carried out in the laboratory to prepare mass culture of a promising isolate *D* of *Dactylaria brochopaga* on different kind of locally available substrates. Based on visual rating on mass culture, production of *Dactylaria brochopaga* was good on barley, sorghum and wheat grain, fair on maize grain and pigeon-pea bran whereas, poor on chickpea bran, chickpea grain and pea bran. Another experiment was also conducted in pots to study the effect mass culture of *D. brochopaga* for biocontrol of root-gall disease of balsam. The application of mass culture at the rate of 10g/kg soil, its undiluted and diluted spore suspension in soil infested of *M. javanica* before planting of balsam seedlings, increased the plant growth parameters significantly and reduced the number of root-galls of balsam plant by 49.60, 44.09 and 25.19% respectively, whereas, mass culture and its undiluted and diluted spore suspensions with cow dung manure reduced root-knots by 87.40, 77.16 and 51.11% respectively. The bioefficacy of the fungus was enhanced significantly when mass culture and its spore suspensions were applied with cow dung manure.

**Key words:** Meloidogyne javanica, Dactylaria brochopaga, spore suspension, cow dung manure, mass culture

**Introduction**  
Balsam (*Impatiens balsamina*) is an annual plant growing to 25-75 cm in height with a thick but soft stem and found throughout the tropical and subtropical part of the world. The species is native to Southern Asia specifically India and Myanmar. *Meloidogyne* spp. is important parasite of many crops and cause economics losses throughout the world (Sasser, 1990; Sikora and Fernandez, 2005; Taylor and Sasser, 1978). Moreover, nematodes are known capable of predisposing plants to the development of complex disease with fungi, bacteria and viruses (Sasser, 1990, Agrios, 2005; Khan, 2010;). The reports of Saba and Khan, (2010) indicated that the association of *Meloidogyne javanica* and *Macrophomina phaseolina* cause disease-complex and severe damage to the balsam plant. *M. javanica* completes its life cycle on balsam plant within 23 days at temperature ranging between 25-30°C (Khan et al., 2006). Five hundred and above second stage juveniles of *M. javanica* is detrimental to balsam plant growth and can significantly decrease the length and dry weight of the plant. (Khan et al., 2006). Nematicides significantly reduce the plant parasitic nematodes but have been too costly for use especially in developing countries, where their uses have been limited to few economic crops (Hague and Gowen, 1987). Due to the above facts researchers are more
interested in biological control of the nematodes in its widest sense, which is also eco-friendly (Fatma A. M. Mostafa, 2001; Amer-Zareen et al., 2003; Kumar and Singh, 2010) and also diseases-complex caused by species of Meloidogyne and fungi (Saba and Khan, 2010). Several attempts have been made to control the activities of plant parasitic nematodes by adding nematophagous fungi to infested soil (Bandyopadhyay et al., 2001; Duddington, 1957; Kumar, 2003; Kumar, 2007; Kumar and Singh, 2006; Kumar and Singh, 2010; Linford et al., 1939; Singh, 2003; Singh, et al., 2006; Singh et al., 2007; Stirling, 1991; Stirling et al., 1998). For addition of the nematophagous fungi in nematode infested soil, there is immediate need for mass production of the fungi on locally available substrates. that the large-scale application of the fungi could been possible. Moreover, it has been observed that the application of organic manures in combination with nematophagous fungi stimulates the bioefficacy of these fungi and consequently, lower the population of root-gall nematodes. (Bandyopadhyay et al., 2001; Kumar, 2003; Kumar and Singh, 2006; Kumar and Singh, 2010; Kumar, 2007; Singh, 2003; Wachira et al., 2009). However, the mechanisms behind stimulated activities of these nematophagous fungi in combination with organic manures were not cleared at that time and are not fully understood till today.

Dactylaria brochopaga is a nematode-destroying fungus, which dramatically captures and kills free-living and parasitic nematodes in vivo and in vitro by producing three celled constricting rings. D. brochopaga is a common fungus in agricultural soils, decomposing plant materials and old decayed root-galls (Saadabi, 2006; Bandyopadhyay, 1998; Kumar et al., 2010; Kumar, 2003; Kumar and Singh, 2010; Singh, 2003; Singh et al., 2007). The bioefficacy of this fungus in reducing the population of M. graminicola was described by Singh et al., (2006) and recently, in reducing the population of M. incognita by Kumar and Singh, (2010).

Materials and Methods

Isolation and mass culture of Dactylaria brochopaga
Five isolates of Dactylaria brochopaga were got from different agricultural soils and decaying substrates from different parts of India by the method described by Duddington (1955) with slight modification (Bandyopadhyay and Singh, 2000). All the five isolates of D. brochopaga were purified by single spore isolation, method given by Singh et al., (2004) and culture of each isolate was maintained at 29±1°C on corn meal agar (CMA) medium by regular subculturing at an interval of 15 days.

To prepare the mass culture of D. brochopaga (Isolate D), several substrates viz., grains of sorghum (Sorghum bicolor), wheat (Triticum aestivum), Chickpea (Cicer arietinum), barley (Hordeum vulgare), maize (Maize zea) and bran of pigeon-pea (Cajanus cajan
L. Millisp), Chickpea (Cicer arietinum) and pea (Pisum sativum L.) were taken separately in 250 ml conical flask. The substrate and water combinations were taken as described by Kumar et al., (2005). The flasks were plugged with cotton and sterilized two times at 15 psi for 20 minutes. Furthermore, the parboiled grains of wheat, barley and sorghum were mixed with CaCO₃ at 10g per kg grains, and taken into 250 ml flasks. The flasks were plugged with cotton and sterilized two times at 15 psi for 20 minutes. 10 mm fungal disc was cut from the edge of the 10 days old culture of the fungus by a sterilized cork borer and inoculated in the centre of flask contained substrate with the help of sterilized inoculation needle. One disc was inoculated in each 250 ml flask. Three replication were maintained for each treatment. The inoculated flasks were incubated at room temperature (25-30°C). Visual ratings were made to assess the growth of D. brochopaga after 25 days of inoculation.

**Biocontrol of root-gall disease of balsam (Impatiens balsamina) by Dactylaria brochopaga**

The experiment on effects of mass culture and spore suspensions of D. brochopaga against root-gall disease of balsam plant was conducted in wire net house of the Department of Mycology and Plant Pathology, Institute of Agricultural sciences, BHU, Varanasi. Root-gall infested sick soil having approximately 1500 second stage juveniles of M. javanica per 1000g soil was used for the experiment. Root-gall infested sick soil was thoroughly mixed by hand to make the uniform population of nematodes before amendments. Mass culture at 1%, its spore suspension (undiluted) and diluted spore suspension (10 times) was amended with or without 5% well decomposed cow dung manure (CDM). Sick soil without mass culture and with cow dung manure served as control. Mass culture, its spore suspension, ten times diluted spore suspension with and without cow dung manure was uniformly mixed in sick soil before filling the pots. Root-gall infested soil and amended sick soil were filled in pots (1000g/pot). Four weeks old, seedlings of balsam plant free from root-gall infection were transplanted in pots. Each pot had a single seedling. For each treatment five pots were used as replicates. The pots were watered regularly. Observation on the plant height, root length, fresh weight of root and shoot, number of galls per plant, number of females, egg masses and second stage juveniles of M. javanica per root system were taken after 4 weeks of planting. The number of females and egg masses per root system, second stage juveniles were estimated by the methods described by Kumar and Singh (2006). Data were statistically analyzed using analysis of variance (ANOVA). Treatment means were compared following Ducan’s multiple range test (Gomez and Gomez 1984)
Results
Promising isolate D of *Dactylaria brochopaga* was selected for mass culture on different substrates with different combinations are presented in (Table. 1). On the basis of visual rating, observations on the growth of the fungus on selected substrates showed good growth on sorghum, barley and wheat grain, whereas, poor growth of the fungus was found on pea and chickpea bran and chickpea grains. Fair growth of the fungus was observed on grains of maize and bran of pigeonpea. Furthermore, the addition CaCO$_3$ to substrates especially, sorghum, wheat and barley grain also stimulated the growth of *D. brochopaga* and rated as excellent as compared to grains alone Table. 1).

The observations on the effect of mass culture and its spore suspensions of *Dactylaria brochopaga* (Isolate D) with and without cow dung manure on number of root-galls, population of *Meloidogyne javanica*, and growth parameters of balsam plant (*Impatiens balsamina*) are presented in Table. 2. The application of mass culture and its undiluted and diluted spore suspensions and without cow dung manure reduced the number of root-galls of balsam plants by 49.60, 44.09 and 25.19% respectively, whereas, mass culture and its undiluted and diluted spore suspensions with cow dung manure reduced root-galls by 87.40, 77.16 and 51.11% respectively. Similarly, the application of mass culture and its undiluted and diluted spore suspensions reduced the number of females by 52.35, 50.00 and 31.76%, of egg masses by 36.12, 27.75 and 21.88% and of juveniles by 49.57, 46.08 and 29.93% respectively. The performance of isolate D of *D. brochopaga* as nematode antagonist was enhanced when mass culture and its undiluted and diluted spore suspensions were applied with cow dung manure. The number of female was reduced by 75.83, 65.29 and 48.82%, of egg masses by 75.84, 71.05 and 39.47% and of juveniles’ by 84.88, 72.92 and 64.72% respectively (Table. 2).

Results further made it was clear that all the growth parameters of balsam plant were significantly enhanced when seedlings were raised in soil infested with 1500 juveniles of *M. javanica* per “1000g” which was amended with mass culture and spore suspensions of the fungus. Moreover, even after the dilution of the spore suspension of mass culture of *D. brochopaga* growth of balsam plant was enhanced significantly than control. The application of mass culture, undiluted and diluted spore suspension without cow dung manure gave the plant height 50.98, 40.47 and 28.57% root length 42.85, 36.00 and 30.43% fresh weight of shoot 55.31, 47.20 and 32.30% and fresh weight root 48.08, 43.75 and 23.50% of balsam respectively, whereas, all the treatments with cow dung manure gave superior plant height 61.53, 56.14 and 44.44% root length 55.55, 51.51 and 40.74% fresh weight of shoot 67.24, 61.86 and 49.51% and fresh weight of root 62.51, 57.17 and 48.27% for balsam plants (Table. 2).
Discussion

Excellent growth of *D. brochopaga* on sorghum, barley and wheat grain along with the supplements CaCO₄ and CaCO₃ in substrates may be attributed to availability of all necessary nutrition for the growth and sporulation of this fungus. It has been reported by several workers that CaCO₃ acts as a supplements and influenced the growth and sporulation of fungi (Zuhair and Shems, 1987; )

The bioefficacy of mass culture and spore suspensions of *D. brochopaga* with and without cow dung manure reduced the number of root-galls and nematode population of *M. javanica* and consequently, enhanced the growth parameters of balsam plants. The enhanced growth of balsam plants and reduced root-galls and the nematode population could be attributed to spores and fungal mycelia of *D. brochopaga* (Kumar and Singh, 2010). *M. javanica* is certainly an important pathogen for plant growth when harbours its pathogenic level in soil (Khan *et al.*, 2006) and a serious agent predisposing to the development of the complex diseases with fungi, bacteria and viruses (Agrios, 2005; Khan, 2010; Sasser, 1990). The well developed roots duly protected at initial stage by capturing and killing of nematodes by the fungus resulted in reduction in *M. javanica* population, root-galls and supported the plant growth as well. The findings are more or less supported by Kumar and Singh (2006) reported that application of *A. dactyloides* reduced the number of root-galls of tomato by 66% and increased the plant growth in pot experiments. The effect of *A. dactyloides* was enhanced when its mass culture was applied in combination with cow dung manure. The other possibilities for the increased effect of *D. brochopaga* may be due to increase in population of saprophytic nematodes which after their capturing and killing might increase the population of the fungus and such conditions help in conversion of saprophytic nature to predacious nature of the fungus. Similarly, Kumar and Singh (2010) also observed that the use of *D. brochopaga* reduced the number of root-galls of eggplants and the population of *M. incognita*, which supports the present work. Furthermore, they also reported that the performance of the fungus as bio-agent was enhanced when its mass culture and spore suspensions were applied with cow dung manure, which reduced the number of root-galls, by 48.5-74.8%, of females by 43.5-64.8%, of egg masses by 38.7-68.8% and of juveniles by 48.1-78.6%. Moreover, diluted spore suspension of the fungus also increased the growth of eggplants and reduced the population of *M. incognita* significantly.

Conclusion

The present study have shown that the mass culture and spore suspensions of *Dactylaria brochopaga* enhanced the growth parameters of balsam plant, reduced the number of galls and the population of *M. javanica*. Also the bioefficacy of *D. brochopaga* was enhanced when its mass culture and spore suspensions were applied in
combination with cow dung manure. Obviously, this practice should be accepted as a good management practice for plant parasitic nematodes in order to avoid the indiscriminate use of inorganic nematicides. Of course, the results would be encouraging only where the nematode population is at pathogenic level in the soil.

Acknowledgment

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References


Table 1: Mass culture of *Dactylaria brochopaga* on locally available substrates and its formulation.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Visual Rating</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barley grain</td>
<td>+++++</td>
</tr>
<tr>
<td>Chickpea grain</td>
<td>++</td>
</tr>
<tr>
<td>Maize grain</td>
<td>+++</td>
</tr>
<tr>
<td>Sorghum grain</td>
<td>+++++</td>
</tr>
<tr>
<td>Wheat grain</td>
<td>+++++</td>
</tr>
<tr>
<td>Barley grain + Calcium carbonate</td>
<td>++++++</td>
</tr>
<tr>
<td>Sorghum grain + Calcium carbonate</td>
<td>++++++</td>
</tr>
<tr>
<td>Wheat grain + Calcium carbonate</td>
<td>++++++</td>
</tr>
<tr>
<td>Chickpea bran</td>
<td>++</td>
</tr>
<tr>
<td>Pea bran</td>
<td>++</td>
</tr>
<tr>
<td>Pigeon-pea bran</td>
<td>+++</td>
</tr>
</tbody>
</table>

Visual Rating:
Excellent = +++++  Good = ++++  Fair = +++  Poor = ++

Table 1: Effect of mass culture and spore suspensions of *Dactylaria brochopaga* (Isolate D) on number of root-galls, population of *Meloidogyne javanica* and growth of balsam plant.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>CDM</th>
<th>Db(10^1)</th>
<th>Db(ss)</th>
<th>Db(mc)</th>
<th>Db(10^1) +CDM</th>
<th>Db(ss) +CDM</th>
<th>Db(mc) +CDM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Knots/Plant</td>
<td>63.5 a</td>
<td>59.0 a</td>
<td>47.5 b</td>
<td>35.5 c</td>
<td>32.0 d</td>
<td>31.0 e</td>
<td>14.5 e</td>
<td>8.0 f</td>
</tr>
<tr>
<td>No. of females/Plant</td>
<td>85.0 a</td>
<td>79.5 a</td>
<td>58.0 b</td>
<td>42.5 c</td>
<td>40.5 c</td>
<td>43.5 c</td>
<td>29.5 d</td>
<td>18.5 e</td>
</tr>
<tr>
<td>No. of eggs/Plant</td>
<td>418.5 a</td>
<td>396.0 a</td>
<td>341.5 b</td>
<td>308.5 c</td>
<td>282.0 d</td>
<td>267.5 c</td>
<td>121.5 e</td>
<td>101.0 f</td>
</tr>
<tr>
<td>No. of juveniles(J2s)</td>
<td>14097.5 a</td>
<td>12890.5 a</td>
<td>9876.5 b</td>
<td>7601.0 c</td>
<td>7108.0 c</td>
<td>4972.5 d</td>
<td>3817.0 d</td>
<td>2130.5 e</td>
</tr>
<tr>
<td>Plant height (cm)</td>
<td>12.5 a</td>
<td>14.0 a</td>
<td>17.5 b</td>
<td>21.0 c</td>
<td>25.5 d</td>
<td>22.5 c</td>
<td>28.5 e</td>
<td>32.5 f</td>
</tr>
<tr>
<td>Root length (cm)</td>
<td>8.0 a</td>
<td>9.5 b</td>
<td>11.5 b</td>
<td>13.5 c</td>
<td>14.0 c</td>
<td>13.5 c</td>
<td>16.5 d</td>
<td>18.0 d</td>
</tr>
<tr>
<td>Fresh weight of Shoot/Plant(mg)</td>
<td>1024.5 a</td>
<td>1087.5 a</td>
<td>1513.5 b</td>
<td>1986.0 c</td>
<td>2292.5 d</td>
<td>2029.5 c</td>
<td>2686.0 e</td>
<td>3128.0 f</td>
</tr>
<tr>
<td>Fresh weight of root/Plant(mg)</td>
<td>927.5 a</td>
<td>1092.5 b</td>
<td>1212.5 c</td>
<td>1649.5 d</td>
<td>1786.5 c</td>
<td>1693.0 d</td>
<td>2166.0 f</td>
<td>2474.5 g</td>
</tr>
</tbody>
</table>

Data with different letters (a, b, c, d, e, f & g) show significant difference of row data among at p≤0.05 according to Duncan’s Multiple Range Test (DMRT).

J2s=juveniles; CDM=cow dung manure; Db= *Dactylaria brochopaga*; mc= mass culture; ss= spore suspension; 10^1= ten time dilution.