Evaluation of bio-efficacy of *Bacillus subtilis* (NBAIMCC-B-01211) against disease complex caused by *Meloidogyne incognita* and *Fusarium oxysporum* f.sp. *vasinfectum* in okra


Indian Institute of Horticultural Research, Hessaraghatta Lake P. O., Bengaluru-560 089, India. 
Department of Entomology, C. S. Azad University of Agriculture and Technology, Kanpur, Uttar Pradesh, India 
E-mail: mrsaobio45@gmail.com

**ABSTRACT:** Field studies were conducted at two different agro-climatic regions of India to assess the bio-efficacy of liquid and talc formulations of *Bacillus subtilis* (Cohn 1872), 1% W.P. for the management of *Meloidogyne incognita* (Kofoid & White, 1919) Chitw, 1949 and *Fusarium oxysporum* f.sp. *vasinfectum* on okra (*Abelmoschus esculentus* Moench.). Results clearly indicated the potential of this bio-pesticide in the management of *M. incognita* and *F. oxysporum* f.sp. *vasinfectum* on okra. *B. subtilis* was enriched in farm yard manure (FYM) and applied at different doses in the experimental plots. Seed treatment with 20g or 20ml of *B. subtilis*/kg and application of five tons of FYM enriched with 5kg or 5 lit *B. subtilis*/ha proved to be significantly effective in reducing the population of *M. incognita* and bringing down the disease incidence of *F. oxysporum* f.sp. *vasinfectum*. These treatments also increased the yield of okra in the two agro-climatic regions.

**Keywords:** *Bacillus subtilis*, *Fusarium oxysporum* f.sp. *vasinfectum*, *Meloidogyne incognita*, management, okra.

**INTRODUCTION**

Intensive farming and excessive chemicalization of agro-ecosystems with various agro-chemicals has affected the soil bio-diversity, adversely tilting the balance towards harmful soil flora and fauna. This has led to the development of a new scenario where in disease complex caused by nematode *M. incognita* and *F. oxysporum* f.sp. *vasinfectum* (Snyder & Hansen, 1940) has assumed a serious dimension in okra (*Abelmoschus esculentus*). Root-knot nematode, *M. incognita* is reported as one of the economically important pests of okra - *A. esculentus* causing 30-50 percent yield reduction (Bhatti and Jain,1977). The nematode makes the plant vulnerable to attack by *F. oxysporum* f.sp. *vasinfectum* (Abuzar and Haseeb, 2006). The yield of okra, tomato and brinjal suffered 90.9, 46.2 and 27.3% loss, respectively, due to *M. incognita* infestation @ 3-4 larvae per gram soil under field conditions in India (Bhatti, 1994).

Rhizobacteria used as seed treatment reduced nematode penetration of the root system in potato and sugar beet (Racke and Sikora, 1992; Oostendorp and Sikora, 1989). The mode of action is thought to be production of metabolites that reduced hatching attraction and degradation of specific root exudates that control nematode behavior (Sikora and Hoffmann-Hergarten, 1993).

Considering the dual importance of this rhizobacteria *B. subtilis* in improving plant growth and disease control, field experiments were carried out to evaluate the bio-efficacy of liquid and talc formulations of *B. subtilis*, the potential bio-pesticide in the management of disease complex on okra caused by *F. oxysporum* f.sp.
vasinfectum and root knot nematode *M. incognita* in two agro-climatic regions of India viz., Bengaluru and Kanpur.

**MATERIALS AND METHODS**

Two locations were selected for the study in India one at Indian Institute of Horticultural Research (IIHR), Bengaluru, Karnataka and the other at C. S. Azad University of Agriculture & Technology (CSAUA&T), Kanpur, Uttar Pradesh. A local isolate of *B. subtilis* IIHR-Bs-2 accession number NBAIMCC-B-01211 (the identification confirmed by molecular characterization) was mass produced at IIHR, Bengaluru.

Experiments were carried out to examine the effect of seed treatment @20 g/kg of seed with talc formulation or 20 ml/kg of seed with liquid formulation of *B. subtilis*. For the field experiments, formulated products of *B. subtilis* 1% W.P. or *B. subtilis* 1% A.S. (CFU 2 x 10\(^8\)/g) were added separately to FYM and left under shade for 15 days with moisture content of 25-28 percent. The experiments were conducted at the different agro climatic regions such as IIHR, Bengaluru, Karnataka (13°58’N, 78° E, and at 890 m above mean sea level) and C.S. Azad University of Agriculture & Technology Kanpur (25°26’ to 26°58’ N, 79°31’ to 80°34’ E and 125.9 m above mean sea level) in sick plots.

Each plot with a size of 4 x 2.5 m² received different treatments as given below. Initial population densities of nematode in soil at the centers were in the range of 129-148 ± 1 per 100 g of soil. Initial population densities of *F. oxysporum* f.sp. vasinfectum were in the range of 4.5x10\(^4\)- 6.5x10\(^4\) CFU/g of soil. The soil in the experimental fields was incorporated with bio-pesticide enriched FYM and one control check was maintained without any treatment. The treatments were replicated ten times each in a randomized block design. The dates of sowing of okra (cv. Solar 10) were 20th July 2012 and July 19th 2013 and dates of termination of the experiment were November 8th 2012 and 15th November 2013 at IIHR, Bengaluru. In kharif season dates of sowing of Okra (var. Pusa Sawani) were 5th July 2012 and 7th July 2013 and date of terminations were 7th November 2012 and 18th November 2013 at (CSAUA&T), Kanpur centre. Three months after sowing data were recorded on root-knot index on a 1-5 scale (Bridge and Page, 1980), densities of *M. incognita* 10 g root, disease incidence and yield/plot. Root populations of the *M. incognita* were estimated from 10 g samples of roots from each plant. The root samples were stained using acid fuchsins following the method of Bridge *et al.* (1982), homogenised, and the numbers of nematodes in the roots were recorded. The experiments were conducted in two seasons during 2012-2013 in these agro-climatic regions. The data were analyzed by using standard statistical methods. The treatments were as follows:

- **T\(_1\)**: Seed treatment with *B. subtilis* 1% @ 20 g/kg of seed or *B. subtilis* 1% A.S. @ 20 ml/kg of seed
- **T\(_2\)**: **T\(_1\)** + 5 tons FYM with 2 kg of *B. subtilis*/ha or 2 lit of *B. subtilis* 1% A.S./ha
- **T\(_3\)**: **T\(_1\)** + 5 tons FYM enriched with 3.5 kg *B. subtilis*/ha or 3.5 lit of *B. subtilis* 1% A.S./ha
- **T\(_4\)**: **T\(_1\)** + 5 ton FYM enriched with 5 kg *B. subtilis*/ha or 5 lit of *B. subtilis* 1% A.S./ha
- **T\(_5\)**: Carbofuran @ 1.0 kg a.i./ha and carbendazim 5kg/ha.
- **T\(_6\)**: Control

**RESULTS AND DISCUSSION**

The results of the field trials showed significant reduction in the infestation of *M. incognita* and disease incidence of *F. oxysporum* f.sp. vasinfectum on okra roots in the treatment where seeds treated at 20g/kg or 20ml/kg and incorporated 5 tons of FYM enriched with 5kg of bio-pesticide *B. subtilis* – 1% W.P. or *B. subtilis* – 1% A.S. (5 l) at both the centers mentioned above (Tables 1-4). These treatments had significantly increased the yield of the crop and are at par with chemical treatment.

Initially, we observed the bio-efficacy of talc and liquid formulation of *B. subtilis* (strain no. IIHR Bs-2) *in vitro* on these target pathogens at both the centers and we did not observe any significant differences in bio-efficacy of these two formulations (unpublished).

Hence, we thought of evaluating both talc and liquid formulation of *B. subtilis* in the field trials at two agro-climatic regions to investigate if there could be any differences in the bio-efficacy of *B. subtilis* in its effect on target pathogens in okra eco-system (Table 1-4).

Seeds are often treated with fungicides for the control of seed-borne and soil-borne diseases (Ramos and Riberio, 1993). Seed treatment is an efficient method for introducing bio-control agents into the soil root environment. Seed coating with microbial antagonists protects the seed from seed-borne and soil borne pathogens and enables the seeds to germinate and become established as healthy seedlings.

The association of plant-parasitic nematodes with a wilt fungus is reported to produce greater loss than
caused by a pathogen alone (Franel and Wheeler, 1993). In our studies also we observed this phenomenon.

Further B. subtilis was reported to promote plant growth by producing growth regulators, including root exudation and enhancing the availability of nutrients to plants besides control of soil-borne pathogens (Weller, 1988). These characteristics make these species good candidates for use as seed inoculants and root dips for biological control of soil-borne plant pathogens. Bacillus spp. have been tested on a wide variety of plant species for their ability to control diseases (Cook and Backer, 1983) and our studies supports these findings.

Application of bio-pesticides to the tune of 20-25 kg/ha (Chaya and Rao, 2012) would be very expensive for the farmer and use of this huge quantity of bio-pesticides is practically not possible. Hence, these investigations were carried out to develop an efficient and effective delivery system for the application of this potential bio-pesticide in different agro-climatic regions in India. FYM was found to be one of the suitable organic materials which could be enriched with this bio-pesticide for the application in the main field.

The dosage of five tons of FYM enriched with 5 kg of B. subtilis - 1% W.P. or B. subtilis – 1% A.S. was significantly effective in reducing the root-knot index and nematode population in the roots and disease incidence caused by F. oxysporum f.sp. vasinfectum (Table 1-4). Application of lower dosages of bio-pesticides was not that effective in bringing down the incidence of disease complex caused by M. incognita and F. oxysporum f.sp. vasinfectum in okra (Table 1-4). Hence, it is important to apply optimum amount of bio-pesticides in the field for getting desirable effect on the target pathogens in the crop eco-system. As such, we have also worked out cost benefit ratio of application of talc and liquid formulation of B. subtilis in okra ecosystems (unpublished).

The experiments were repeated in two seasons during the year 2012 to 2013 to confirm the results on

Table 1. Bio-efficacy of B. subtilis 1% W.P. against disease complex caused by M. incognita and F. oxysporum f.sp. vasinfectum infecting okra (Kanpur)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Root knot index (1-5)</th>
<th>No. of M. incognita 10 g. of root</th>
<th>Disease incidence (%)</th>
<th>Yield/plot (kg)</th>
<th>Increase in yield (%)</th>
<th>Root colonization (CFU/g of Root) of B. subtilis x10⁶</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>3.0</td>
<td>3.1</td>
<td>24</td>
<td>22</td>
<td>33.2</td>
<td>32.4</td>
</tr>
<tr>
<td>T2</td>
<td>2.5</td>
<td>2.4</td>
<td>19</td>
<td>18</td>
<td>22.3</td>
<td>25.1</td>
</tr>
<tr>
<td>T3</td>
<td>2.0</td>
<td>2.3</td>
<td>12</td>
<td>13</td>
<td>17.4</td>
<td>18.8</td>
</tr>
<tr>
<td>T4</td>
<td>1.8</td>
<td>1.9</td>
<td>10</td>
<td>12</td>
<td>8.5</td>
<td>8.1</td>
</tr>
<tr>
<td>T5</td>
<td>1.3</td>
<td>1.5</td>
<td>15</td>
<td>17</td>
<td>12.5</td>
<td>12.7</td>
</tr>
<tr>
<td>T6</td>
<td>3.9</td>
<td>4.2</td>
<td>26</td>
<td>28</td>
<td>36.1</td>
<td>29.4</td>
</tr>
<tr>
<td>CD @ 5%</td>
<td>0.28</td>
<td>0.35</td>
<td>3.48</td>
<td>2.8</td>
<td>5.43</td>
<td>4.22</td>
</tr>
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</table>

Table 2. Bio-efficacy of B. subtilis 1% W.P. against disease complex of okra (IIHR, Bengaluru)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Root knot index (1-5)</th>
<th>No. of M. incognita 10 g. of root</th>
<th>Disease incidence (%)</th>
<th>Yield/plot (kg)</th>
<th>Increase in yield (%)</th>
<th>Root colonization (CFU/g of Root) of B. subtilis x10⁶</th>
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<td>3.3</td>
<td>23</td>
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<td>26.9</td>
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<tr>
<td>T2</td>
<td>2.8</td>
<td>2.7</td>
<td>18</td>
<td>17</td>
<td>19.6</td>
<td>20.7</td>
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<tr>
<td>T3</td>
<td>2.3</td>
<td>2.0</td>
<td>13</td>
<td>15</td>
<td>15.9</td>
<td>17.3</td>
</tr>
<tr>
<td>T4</td>
<td>1.7</td>
<td>1.6</td>
<td>7</td>
<td>6</td>
<td>6.3</td>
<td>6.9</td>
</tr>
<tr>
<td>T5</td>
<td>2.1</td>
<td>2.2</td>
<td>14</td>
<td>16</td>
<td>10.2</td>
<td>9.8</td>
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<tr>
<td>T6</td>
<td>4.2</td>
<td>3.8</td>
<td>26</td>
<td>29</td>
<td>28.6</td>
<td>33.4</td>
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<tr>
<td>CD @ 5%</td>
<td>0.38</td>
<td>0.57</td>
<td>1.84</td>
<td>2.23</td>
<td>1.76</td>
<td>2.15</td>
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</table>

the efficacy of application of this bio-pesticide enriched FYM on the management of disease complex on okra under the field conditions in two different climatic regions. The findings clearly indicate the potential use of \textit{B. subtilis} 1% W.P. and \textit{B. subtilis} 1% A.S enriched FYM for the bio-management of disease caused by \textit{M. incognita} and \textit{F. oxysporum} f.sp. \textit{vasinfectum} on okra under field conditions. Further the data also clearly establish the nematicidal as well as fungicidal properties of \textit{B. subtilis} for the first time in the world. On the basis of the data, we conclude that talc and liquid formulation of \textit{B. subtilis} are equally effective in the management of okra disease complex in south as well as north Indian conditions.

ACKNOWLEDGEMENT

The authors thank the Director, IIHR, Bengaluru and the Director of Research, C. S. Azad University of Agriculture & Technology, Kanpur (Uttar Pradesh), for facilitating these field trials.

REFERENCES


Bioefficacy of *Bacillus subtilis*


*MS Received : 10 November 2014*

*MS Accepted : 23 December 2014*