The integration efficacy of formulated abamectin, *Bacillus thuringiensis* and *Bacillus subtilis* for managing *Meloidogyne incognita* (Kofoid and White) Chitwood on tomatoes

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**ABSTRACT**

A pot trial was conducted to evaluate the impact of formulated abamectin, *Bacillus thuringiensis* and *Bacillus subtilis* alone or in combinations against the root-knot nematode (*Meloidogyne incognita*) development in tomato plants. Abamectin was the superior treatment which reduced galls and soil populations by 85.87 and 85.13%, respectively. The single treatments showed that the high rate of *B. thuringiensis* and or *B. subtilis* recorded the highest reductions than the low rate. The single treatments of *B. thuringiensis* and *B. subtilis* gave reductions in galls ranged from 66.22 to 78.88%, and in soil populations ranged from 70.63 to 80.45%. Moreover, the binary mixtures of abamectin + *B. thuringiensis* recorded the highest reductions among mixtures in galls (85.20%) and soil populations (76.56%). On the other side, all the applied single treatments increased the tomato plant growth criteria over control. The binary blend of abamectin + *B. thuringiensis* recorded the highest augmentation in root length, root fresh weight and shoot fresh weight by 41.84, 49.58 and 56.72%, respectively.

**Keywords:** *Meloidogyne* sp., Abamectin, *Bacillus thuringiensis*, *Bacillus subtilis*, Integrated Nematodes Management.

**INTRODUCTION**

Tomato is a crop affected by certain types of pests and/or diseases which decreased the quality and quantity of production. In Egypt, tomato is considered one of the most important vegetable crops for fresh consumption and processing (Abd El-Ghany, 2011). Tomatoes are mainly used as rich source of carbohydrates, minerals and vitamins (Ibrahim *et al*., 2010). One of the main pests in tomato crop is the root-knot nematodes, *Meloidogyne* spp., which are responsible for more than 27% yield losses in tomato globally (Sharma and Sharma, 2015).

Research papers and reports for many organizations have established he hazards of pesticides on the human life and his surrounding environment. It has become necessary to find alternative approaches to synthetic nematicides. Abamectin which is a mixture of macrocyclic lactone metabolites produced by the bacterium *Streptomyces avermectinis* is an effective alternative against plant parasitic nematodes according to other investigations of Huang *et al*., (2014), El-Nagdi *et al*., (2015) and Saad *et al*., (2017).

Furthermore, *Bacillus thuringiensis* Berliner is one of the untraditional microbes used against different genera of plant nematodes especially root-knot nematodes. *B. thuringiensis* produce parasporal crystalline proteinaceous inclusions or δ- endotoxins which are toxic to parasitic nematodes (Kotze *et al*., 2005; Iatsenko *et al*., 2014). Furthermore, *Bacillus subtilis*, is one of the famous plant growth promoting rhizobacteria
(PGPR), which attracted global attention as a bio-control agent in the management of plant pathogenic nematodes (Prakob et al., 2009 and Xiang et al., 2017). It was found that B. subtilis improved the plant growth indices significantly (Khalil et al., 2012). Therefore, this study aimed to evaluate the efficacy of abamectin, B. thuringiensis and B. subtilis alone and / or in combinations against the root-knot nematode (Meloidogyne incognita) as alternative solutions to chemical control problem on tomato.

METRIALS AND METHODS

Identification of root-knot nematode

The eggs of the root-knot nematode, Meloidogyne incognita (Kofoid and White) Chitwood, were isolated from infested roots of eggplant (Solanum melongena) with sodium hypochlorite (NaOCl) according to the method of Hussey and Barker (1973). The eggs suspension was passed through a 200 mesh sieve and collected on a 400 mesh sieve to obtain free eggs before carrying out the experiment. The species of the root-knot nematode (Meloidogyne incognita) was identified by using the perineal patterns method according to Taylor and Nelscher (1974).

Tested materials and their rates of applications

Dipel® 54% DF containing 32,000 iu/mg of bacterium Bacillus thuringiensis var. kurstaki. It was applied at 5 and 10 g / kg soil

Sting® containing 1×10⁹ cell/ ml of bacterium Bacillus subtilis. It was applied at 5 and 10 ml / kg soil.

Tervigo® 2% SC (Abamectin). It was applied in the recommended dose 2.5 L / feddan.

The experimental design

Pots experiment was carried out using tomato plants cv. YARA, each pot were filled with 1kg of loamy sand soil. Five weeks old tomato seedlings were transplanted and infected with the eggs suspension of Meloidogyne incognita which applied at the rate of 5000 eggs / pot by making holes around the root system at uniform distance. Nine treatments were applied and each treatment was replicated five times, and each replicate contains one plantlet. Sixty days after planting, the seedlings were uprooted and assessed for the shoot and root lengths and fresh weights. Meanwhile, the numbers of J₂ populations' were evaluated in the soil (250 g) using the sieving and Baermann plates’ technique (Ayoub, 1980), in addition to galls number on plant roots.

The applied treatments were; Meloidogyne incognita only (Untreated check), M. incognita + Abamectin (Recommended dose), M. incognita + Bacillus thuringiensis (at 5g / kg soil), M. incognita + Bacillus thuringiensis (at 10g / kg soil), M. incognita + Bacillus subtilis (at 5ml / kg soil), M. incognita + Bacillus subtilis (at 10ml / kg soil), M. incognita + Abamectin(at half dose) + B. thuringiensis (at 5g/ kg soil), M. incognita + Abamectin(at half dose) + B. subtilis (at 5ml/ kg soil) and M. incognita + B. subtilis (at 5ml/ kg soil) + B. thuringiensis (at 5g/ kg soil).

Statistical analysis

The pots were arranged in complete randomized design (CRD). The least significant differences (LSD) at the 5% level of probability were determined using a computer program Costat Version: 6.303 (2005).

RESULTS AND DISCUSSION

The biological performance of formulated abamectin, Bacillus thuringiensis and Bacillus subtilis, alone and in combinations was evaluated against galls formation and soil population density of Meloidogyne incognita infecting tomato plants (Table 1). The single treatments showed that abamectin, B. thuringiensis and B. subtilis both at high rates recorded the reductions in galls by 85, 78.88 and 71.02%, respectively. However, the low rate of B. thuringiensis and B. subtilis recorded the least reductions in galls formation by 68.57 and 66.22%, consecutively. The combined treatments showed that the binary mixture between abamectin + B. thuringiensis, abamectin + B. subtilis and B. thuringiensis + B. subtilis recorded decrement in galls by 85.20, 75.92 and 68.16%, respectively.

On the other hand, all the applied treatments exhibited decreasing in soil populations’ density significantly over control. The results in Table 1 indicated that abamectin alone...
The effect of abamectin, *Bacillus thuringiensis* and *Bacillus subtilis* alone and in combinations against *Meloidogyne incognita* in tomato plants.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Rate (ml or g / kg soil)</th>
<th>Galls / root system</th>
<th>Soil populations / 250g soil</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean ± SE</td>
<td>% decrease in galls over control</td>
</tr>
<tr>
<td>Abamectin</td>
<td>0.0025 ml</td>
<td>27.70±2.72</td>
<td>85.87</td>
</tr>
<tr>
<td><em>Ba. thuringiensis</em></td>
<td>5g</td>
<td>61.60±6.17</td>
<td>68.57</td>
</tr>
<tr>
<td></td>
<td>10g</td>
<td>41.40±5.77</td>
<td>78.88</td>
</tr>
<tr>
<td><em>Ba. subtilis</em></td>
<td>5 ml</td>
<td>66.20±3.18</td>
<td>66.22</td>
</tr>
<tr>
<td></td>
<td>10 ml</td>
<td>56.80±4.88</td>
<td>71.02</td>
</tr>
<tr>
<td>Abamectin + <em>B. thuringiensis</em></td>
<td>0.0013 ml + 5g</td>
<td>29.00±3.81</td>
<td>85.20</td>
</tr>
<tr>
<td>Abamectin + <em>B. subtilis</em></td>
<td>0.0013 ml + 5 ml</td>
<td>47.20±4.53</td>
<td>75.92</td>
</tr>
<tr>
<td><em>B. thuringiensis</em> + <em>B. subtilis</em></td>
<td>5g + 5 ml</td>
<td>62.40±4.46</td>
<td>68.16</td>
</tr>
<tr>
<td>Untreated Check</td>
<td>-----</td>
<td>196.00±6.14</td>
<td>-----</td>
</tr>
<tr>
<td>LSD 0.05</td>
<td>-----</td>
<td>13.48</td>
<td>-----</td>
</tr>
</tbody>
</table>

Within a column, numbers followed by different letter(s) are significantly different using LSD at p = 0.05.

It was observed that the high rate of *B. thuringiensis* and/or *B. subtilis* was more effective than the low rate. The binary mixture between abamectin + *B. thuringiensis* and abamectin + *B. subtilis* recorded the same significance in galls and soil population reductions. Also, the binary mixture of *B. thuringiensis* + *B. subtilis* did not significantly differ from using each alone in the case of galls reduction.

The present investigation was confirmed by previous studies which proved that abamectin is an effective nematicide against root-knot nematodes (*Meloidogyne* spp.), in different crops (Huang et al., 2014; El-Nagdi et al., 2015 and Saad et al., 2017). It was reported that abamectin diminished tomato root galling and the soil population significantly (Khalil et al., 2012). While Saad et al. (2017) found that abamectin relatively suppressed the galls, egg masses, eggs / mass and soil population of *Meloidogyne incognita* in tomato plants by 66.69, 66.31, 16.34 and 75.34%, respectively.

Abamectin is a new generation of nematicides that considered a new tool in the field of plant parasitic nematodes management. Abamectin have a unique mode of action in comparison with traditional nematicides. Abamectin is targeted the δ- amino-butyric acid (GABA) receptors causing increments in the permeability of chloride ions which finally causing death (Martin et al., 2002). Also, the effect of abamectin as a nematicide is attributed to the strong adsorption to soil particles and the immobility in the soil (Lopez-Perez et al., 2011; Muzhandu et al., 2014). The stability of abamectin in soils leads to more exposure periods of the plant nematodes to the compound. The commercial products of *Bacillus thuringiensis* have proved the ability for managing the root-knot nematode (*Meloidogyne* spp.) in different investigations (Radwan, 2007; Khalil et al., 2012; Khalil,
pathogenic nematodes belonging to the genera *Heterodera* and *Meloidogyne*, included *B. amyloliquifaciens*, *B. cereus*, *B. licheniformis*, *B. megaterium* and *B. thuringiensis*. Bacteria are suggested to produce metabolites which reduce hatch and attraction and/or degradation of specific root exudates which control nematode behavior (Sikora and Hoffmann-Hergarten, 1993).

As for the bio-based product of the bacterium, *B. subtilis*, the present results complement those of Khalil et al., (2012) and Khalil (2013) who found that *B. subtilis* was effective against the galls and egg masses of *M. incognita* in tomato plants and population density in the soil. In micro-plots trial, the *B. subtilis* strains significantly reduced the number of eggs/g roots with values ranged from 73.63 to 80.72 % after 48 days of application on cotton and increased cotton yield (Xiang et al., 2017). Also, Mohamedova and Samaliev (2011) tested the nematocidal activity of two local strains of *B. subtilis* applied to the soil at different time intervals. The application of *B. subtilis* significantly reduced the developmental stages, females, egg masses and eggs/ mass of *M. arenaria* on roots of potato plants.

The reduction of plant parasitic nematodes associated with *B. subtilis* as a plant growth promoting rhizobacteria (PGPR) may be attributed to diverse mechanisms which involve phytohormones production, mineral solubilization, reduction of the activity of egg hatching factors, alteration of root exudates and inhibition of nematode penetration into the roots as well as reducing galling (Karanja et al., 2007). Furthermore, several reports clarified that the basic mechanisms of *B. subtilis* included direct parasitism, production of extracellular antibiotics or other substances, enhance plant growth, induce systemic resistance in plants, managing the plant diseases and secreting volatile nematocidal products (Ji et al., 2006 and Huang et al., 2009). On the other hand, the effect of applied treatments on the tomato growth indices was recorded in Table 2. The root system length was recorded and the best single treatment was...
In the same context, the shoot length of tomato plants was increased with the single treatment of *B. thuringiensis* at high rate, *B. thuringiensis* at low rate, *B. subtilis* at high rate, *B. subtilis* at low rate and abamectin by 28.99, 24.95, 18.11, 15.26 and 8.11%, respectively. The binary blend of abamectin + *B. thuringiensis*, abamectin + *B. subtilis* and *B. subtilis* + *B. thuringiensis* increased the root length significantly by 41.84, 18.37 and 9.18%, respectively.

Regarding the root system fresh weight; it was increased with the sole treatments of *B. thuringiensis* at the high rate, *B. thuringiensis* at low rate, *B. subtilis* at high rate, *B. subtilis* at low rate and abamectin by 29.89, 24.95, 18.11, 15.26 and 8.11%, consecutively. The binary blends of abamectin + *B. thuringiensis*, *B. subtilis* + *B. thuringiensis* and abamectin + *B. subtilis* achieved augmentations reaching up to 49.58, 23.26 and 17.26%, respectively.

In the same context, the shoot length of tomato plants was increased significantly with the single treatment of *B. subtilis* at high rate with 20.78%, followed by *B. thuringiensis* at high rate, abamectin, *B. thuringiensis* and *B. subtilis* both at low rate with augmentations by 20.00, 16.96, 11.30 and 2.17%, respectively. While the binary mixture of abamectin + *B. subtilis*, *B. subtilis* + *B. thuringiensis* and abamectin + *B. thuringiensis* were recorded increasing by 23.48, 13.91 and 9.57%, consecutively.

Besides, the shoot system fresh weight was also increased without significant differences by 39.95, 33.17, 27.20, 22.73 and 18.27% in pots received the single treatments of *B. thuringiensis* at high rate, abamectin, *B. thuringiensis* at low rate, *B. subtilis* at high rate and *B. subtilis* at low rate, respectively. The mixed treatments of abamectin + *B. thuringiensis*, abamectin + *B. subtilis* and *B. subtilis* + *B. thuringiensis* were gave the highest augmentations by 56.72, 56.40 and 36.58%, respectively.

The gained results are in conformity with those obtained by Radwan (2007), Khan et al. (2010) and Khalil (2013) who found that *B. thuringiensis* increased the shoot system indices significantly. In addition to, abamectin enhanced the shoot and root length and fresh weight of tomato plants (Ibrahim et al., 2010; Khalil et al., 2012; Saad et al., 2012; Muzhandu et al., 2014).

In certain investigations it were found that the plant growth characteristics as shoot and root system were increased significantly in the presence of *B. subtilis* (Khalil et al., 2012; Khalil, 2013; Xiang et al., 2017). Also, the using of *P. fluorescens* and *B. subtilis*, singly and in mixture against *M. incognita* ameliorating the plant growth criteria of onion crop (Munshid et al., 2013).

To date, in Egypt, the farmers are still depending on chemical nematicides. But the adverse effects on human health were noticed.
Therefore, in this study we try to study alternative tools such as abamectin that registered newly in Egypt, _B. thuringiensis_ and _B. subtilis_ alone or in combinations as safe suggestions for the integrated nematodes management programs (INMP).

**REFERENCE**


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Biopesticide of Mirabilis jalapa against S. litura


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