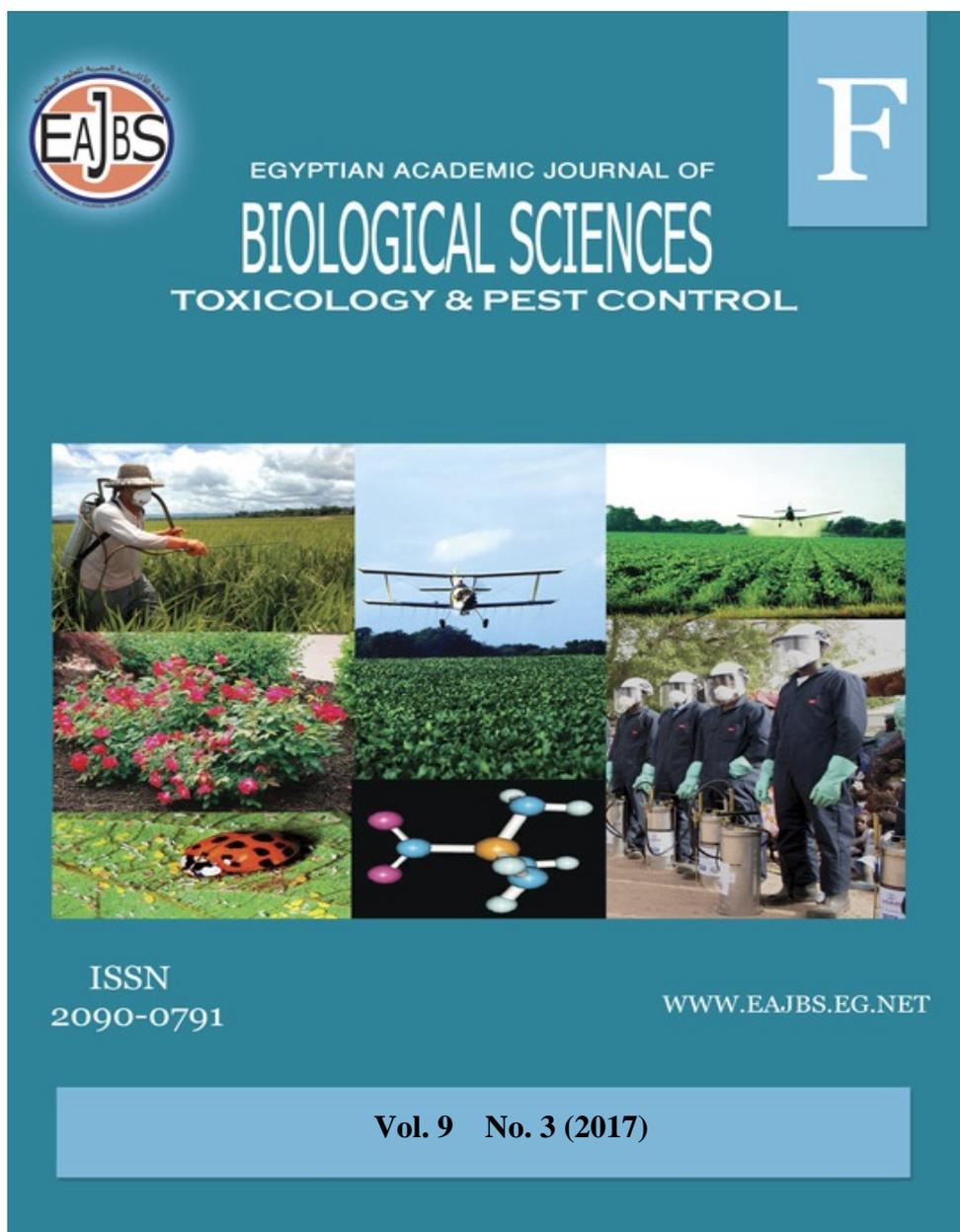


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Control of Root Lesion Nematode *Pratylenchus* spp. Infesting Potato Cv. Spunta Under Field Conditions Through Biotic Products.

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ABSTRACT

In Egypt potato is the most popular solanaceous vegetables either for local consumption and exportation. Previous investigations showed that potato have been attacked by many plant parasitic nematodes. The root-lesion nematodes (*Pratylenchus* spp.) are economically the most important plant pathogens after root knot nematodes. They cause an average growth inhibition of 59.6% of infected potato seedlings with losses in tuber yields of 20-50% and in total plant weight of 50%. Producers have relied mainly on nematicides and chemical fertilizers to control plant parasitic nematodes and improved soil fertility, but their applications are associated with myriads of problems on human health and environment. Due to the consumer demand for chemicals-free food the main goal of this work is to evaluate the potentialities of some commercial bioproducts in controlling root lesion nematodes *Pratylenchus* spp. infecting potato cv. Spunta and improving yield production, under field conditions. The bioagents that have been assessed and their commercial name are Microbien containing the N₂ fixing bacteria *Pseudomonas* spp. and *Bacillus megatherium*, Potassiumag containing the potassium solubilizing bacterium *Bacillus circulans* and Phosphorine contain the phosphorus solubilizing bacterium *Bacillus megatherium*, Nemafree containing *Serratia* spp., the Stanes Symbion VAM Plus containing the vascular arbuscular miccorrhiza *Glomus fasciculatum* and the Stanes Sting containing the rhizobacteria *Bacillus subtilis*. A field experiment was conducted at Kafr-kandeel village, Giza governorate, Egypt to investigate the integration effects between these products to control *Pratylenchus* pp. and improve potato yield production cv. Spunta. The combined treatments are 1-Biofertilizers (Microbien, Phosphorine, Potassiumag) 2- Biofertilizers plus stanes Symbion, 3- Biofertilizers plus Nemafree 4- Biofertilizers, plus Stanes Sting 5- untreated plant using as control. Obtained results after three months showed that all the tested combinations reduced *Pratylenchus* spp. both in soil and roots by different rates, as well as increased potato yield production as compared to untreated control treatment. The highest increase in potato yield production 30% over control resulted from the application of the bionematicides Stanes Sting containing *Bacillus subtilis* in combination with the biofertilizers Microbien, Phosphorine and Potassiumag.

INTRODUCTION

Potato, *Solanum tuberosum* L. is a good source of carbohydrates. Root-lesion nematodes of the genus *Pratylenchus* are recognized worldwide as one of the major constraints of potato cultivation mainly in light soil (Phillis, 1995). These nematodes are migratory endoparasite, moving in and out of the host plant roots, leaving a trail of necrotic tissue in its walk causing an average growth inhibition of 59.6% of infected potato seedlings with losses in tuber yields of 20-50% and in total plant weight of 50% (Bernard and Laughlin, 1976).

In addition, association of these nematodes with other soil borne pathogens caused more economic losses to agricultural crops (Powell and Nusbaum, 1960). Chemical nematicides have been the primary management tool of plant parasitic nematodes for over fifty years but their applications are associated with myriads of problems. Consequently, chemical control may no longer be a good option (Ploeg, 2002). The recent drive to produce vegetables free of chemical residues and increased concern for the environment and human health led to innovate and develop alternative control approaches which are environmentally safe. One possible alternative is the use of biocontrol strategies which involve the application of microbes antagonistic to nematodes.

The main goal of this work is to evaluate the potentialities of some microorganisms which known to have potential as biocontrol and biofertilizer agents against nematodes in their commercial products (Stanes Sting contains *Bacillus subtilis*, Nemafree contains *Serratia spp.* and Stanes Sambion contains the vesicular-arbuscular mycorrhizal *Glomus fasciculatum*, and that play as biofertilizers Microbien contain the N₂-fixing bacteria *Pseudomonas spp.* and *Bacillus megatherium*, Phosphorine contains the phosphate solubilizing bacteria *Bacillus megatherium* and Potassiumag contains potassium solubilizing bacteria *Bacillus circulanes* to manage potato cv. Spunta production

grown in field naturally infested with root lesion nematode *Pratylenchus spp.*

MATERIALS AND METHODS

Field experiments were carried out at Kafr-kandeel village, Giza governorate to evaluate the effectiveness of certain commercial bionematicides combined with some biofertilizers for controlling root lesion nematode *Pratylenchus spp* on potato cv. Spunta. The chosen field area was naturally infested with *Pratylenchus spp.* The experimental field was divided into plots, each of 6m long, 1m apart and the distance between hills was 0.5m where potato eyes were planted in rows. Bionematicides and biofertilizers combined treatments were designed as shown in Table (1) and each one was replicated four times in completely randomized block design. Tested formulations were applied, as labeled using rate equivalent to field application rate. Soil population densities of *Pratylenchus spp.* were initially determined prior to planting time and at the end of the growing season of such treatments. Each treatment was represented by ten compressed samples each comprised three samples. Nematode extraction and counting were done as described by (Barker, 1985). Means of the ten compressed samples were used to calculate the numbers of nematode population per 1kg soil, for each treatment. At harvest time; three months after application, potato tubers were hand-harvested and yield was determined as tons /Feddan, for each treatment and the nematode final population were expressed as nematodes/kg soil.

Table 1: Different treatments of bionematicides and biofertilizers for controlling *Pratylenchus spp.* on potato cv. Spunta under field conditions.

Treatments	
1	Untreated control
2	Microbien, Phosphorine, Potassiumag (Biofertilizers)
3	Biofertilizers plus Stanes Symbion
4	Biofertilizersplus Nemafree
5	BiofertilizersplusStanes Sting

Plants were carefully dug out and gently washed to remove the adhering soil particles from the root surface. Excess water was removed by blotting-paper before weighing shoots and roots separately. Nematode population from 5g roots was extracted and counted as mentioned by (Southey 1970).

Percentage nematode reduction in soil was determined according to Henderson and Tilton formula (Puntener, 1981) as follows.

$$\text{Nematode population reduction (\%)} = \left[1 - \left(\frac{PTA}{PTB} \times \frac{PCB}{PCA} \right) \right] \times 100$$

Where: PTA=

Population in the treated plot after application, PTB= Population in the treated plot before application, PCB= Population in the check plot before

application and PCA= Population in the check plot after application.

RESULTS

Table (2) illustrates the effects of the aforementioned bioproducts in controlling the lesion nematodes *Pratylenchus* spp. infecting potato cv. Spunta under field conditions. Data revealed that all treatments reduced *Pratylenchus* spp. numbers in soil and roots with different rates as compared with the untreated control. At harvest time (three months after application) the application of biofertilizers with *B. subtilis* exhibited the highest efficacy in reducing *Pratylenchus* spp soil population (91.7%) followed by 89.98% in soil treated by biofertilizers as compared with the untreated control plots.

Table 2: Efficacy of certain commercial bioproducts in controlling nematode population of the root lesion nematode *Pratylenchus* spp. infecting potato cv. Spunta under field conditions.

Treatments	Population densities of <i>Pratylenchus</i> spp.				
	Initial population	Final population	% *Red. Efficacy	Number of <i>Pratylenchus</i> spp. in 5g roots	% Red.
Untreated control	1001	759		26	
Biofertilizers(Microbien+ Phosphorine+ Potassuimag)	978	75	89.98	16	38.46
Biofertilizers +Stanes Symbion	932	105	85.14	17	34.62
Biofertilizers +Nemafree	985	175	76.75	17	34.62
Biofertilizers + Stanes Sting	960	65	91.7	16	38.46

*Percentage reduction in soil population of *Pratylenchus* spp. according to Henderson &Tilton Formula Puntener, 1981.

*Efficacy of treatment in reducing nematode population of *Pratylenchus* spp.

The mentioned treatments reduced *Pratylenchus* spp. roots population at the end of the growing season (Table 3) by38.46%. All studied combinations affected positively potato yields production by different rates (Table 3)the application of biofertilizers plus *B. subtilis* induce 30% increase over control followed by 20% dueto the biofertilizers plus *Serratia* spp. Results showed that not all combinations exhibited an increase in weights of potato roots. The highest percentage increase 20% was

achieved by the application of biofertilizers followed by 11% increase with the application of biofertilizers plus *B. subtilis*. All combinations under investigation enhanced potato shoot weights more than the untreated control plants with varied rates (Table 3). Results are in agreement with (Bevivino *et al.*, 1998) who reported that rhizobacteria can stimulate plant growth directly by producing growth hormones and improving nutrient uptake or indirectly by changing microbial balance in the

rhizosphere in favour of beneficial microorganisms. The remarkable reduction in nematode population with the application of *Bacillus subtilis* was explained with more than one mechanism produce the antibiotic bulbiformin directly affect nematodes, also (Qihong *et al.*, 2006) found that the extracellular cuticle-degrading proteases were involved in the processes to penetrate nematode cuticle and eventually digest them and improving plant growth by

solubilizing phosphate and other nutrients and making them available to the plants (Gaur, 1990). Mercer *et al.*, 1992 found that chitinase from *Serratia marcescens* induced premature hatch of nematode eggs. Kamensky *et al.*, 2003 stated that inhibitory metabolites produced by *Serratia* isolates including serrawettin as well as chitinase and other cell wall and cell-membrane degrading enzymes had significant suppressive effect on nematodes.

Table 3: Effect of certain commercial bionematicides and biofertilizers on plant growth response and yield of potato cv. Spunta infected with *Pratylenchus* spp. under field conditions.

Treatments	Root Weight/ plant g	% Incr.	Shoot Weight/ Plant g	% Incr.	Yield/ Feddan Ton	% Incr.
Untreated control	9	—	53	—	7000	—
Biofertilizers(Microbien+ Phosphorine+ Potassuimag)	10.8	20	70	32.1	7700	10
Biofertilizers +Stanes Symbion	7.4	—	69	30.2	7700	10
Biofertilizers +Nemafree	8.8	—	74	39.6	8400	20
Biofertilizers + Stanes Sting	10	11	64	20.8	9100	30

It is clear from Table 2 that *Pratylenchus* spp. are much more found in soil than in roots at harvest time this is refer to general trend of lesion nematodes, where many reniform *Pratylenchus* individuals leave the roots and enter the soil at the end of the growing season of the annual crops and remain until a new crop is planted as reported by Timper and Brodie, 1993.

Results showed that treated soil with the three biofertilizers; Microbien, Phosphorine and Potassium mag containing each of the rhizobacteria *Pseudomonas* pp., *B. megatherium*, *B. circulanes* that had involved in the biological processes of N₂ fixation Nitrogen and solubilizing phosphate and potassium improved plant production and gave a remarkable reduction in nematode population. The same results were discussed in previous studies of Kirkpatrick *et al.* (1964) who reported that the greater availability of phosphorus made the host plant strong enough to tolerate pathogen attack and also phosphorus itself played a vital role in building self-defense of plants against

nematodes. Al-Rehiayani *et al.*, 1999 found that *B. megatherium* reduced penetration of *Pratylenchus penetrans* in potato by 50%, Padgham and Sikora, 2007 stated that treatment with *B. Megatherium* resulted in a greater than 40% reduction in nematode penetration. Also Siddiqui and Akhtar, 2007 reported that Phosphate-solubilizing microorganisms have potential for the biocontrol of plant pathogens as they change insoluble phosphatic compounds into soluble forms thus increasing the growth and yield of crop plants. The Arbuscular mycorrhizal fungi (AMF) *Glomus fasciculatum* are endophytic fungi that grow within plant tissues without causing disease and can play a protective role against parasitic nematodes by forming distinct symbiotic structures and are of great value in promoting the uptake of phosphorus, minor elements and water (Allen, 1996 and Ibijbijen *et al.*, 1996). They also influence the severity of several plant pathogens by altering plant-pathogen interactions (Akkopru and Demir, 2005).

We can conclude that two or more biopreparations in such treatment give potential to the biological control of plant parasitic nematode and produce food free from chemical residues.

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ARABIC SUMMERY

مكافحة نيماتودا التفرح الجذري جنس براتيلنكس التي تصيب نباتات البطاطس صنف اسبونتا تحت الظروف الحقلية باستخدام بعض المركبات الحيوية.

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يعتبر محصول البطاطس من أهم محاصيل الخضر التي تتبع العائلة الباذنجانية لما له من أهمية اقتصادية كبيرة سواء للاستهلاك المحلى او التصدير. تعتبر نيماتودا التفرح الجذري من اهم الافات التي تسبب ضررا لمحصول البطاطس فى مصر بعد نيماتودا تعقد الجذور. و قد اعتمد المنتجين لفترة طويلة على المبيدات الكيميائية لمكافحة هذه الافات مع الاسمدة الكيميائية لخصوبة التربة مما تسبب فى العديد من المشاكل الناتجة من متبقيات هذه الاضافات الكيميائية الكثيرة سواء على صحة الانسان و الحيوان أو البيئة. و مع زيادة الطلب على المنتجات الزراعية الخالية من آثار المواد الكيماوية دفع ذلك العلماء للبحث عن بدائل آمنة لمقاومة الافات النيماتودية وتحسين المحصول. فكان الغرض من هذه الدراسة هو تقييم فاعلية بعض المنتجات الحيوية التجارية لمكافحة نيماتودا التفرح الجذري التي تصيب نباتات البطاطس صنف اسبونتا و زيادة المحصول تحت ظروف الحقل. كانت المركبات الحيوية المستخدمة هي المخصبات الحيوية ميكروبيين المثبتة للنتروجين الجوى، فوسفورين الميسره للفوسفور و بوتاسيوماج الميسره للبتواسيوم التي تحتوى على البكتريا بسيدومونس فلوريسنس مع باسيلس ميجاتيرم، البكتريا باسيلس سيركولينس ، البكتريا باسيلس ميجاتيرم على التوالي المبيدات الحيوية نيمافرى المحتوى على البكتريا سريشيا، استنس استنتاج المحتوى على البكتريا باسيلس ساتلس و استنس سابيون المحتوى على الفطر الوعائى جلوماس اسيكوليتم. و قد تم تقييم هذه المركبات الحيوية فى حقل مصاب طبيعيا بنيماتودا التفرح الجذري جنس براتيلنكس فى قرية كفر قنديل محافظة الجيزة جمهورية مصر العربية عن طريق المعاملات التالية ١-الثلاث اسمدة الحيوية ميكروبيين، فوسفورين و بوتاسيوماج ٢- الثلاث اسمدة الحيوية و استنس سابيون ٣- الثلاث اسمدة الحيوية مع نيمافرى ٤-الثلاث اسمدة الحيوية مع استنس استنج ٥- بالاضافة الى معاملة المقارنة بدون أى اضافات. و قد أظهرت هذه المعاملات قدرة هذه المركبات على تقليل اعداد نيماتودا التفرح الجذري فى التربة و الجذور مع زيادة فى محصول البطاطس مقارنة بالكنترول. وكانت افضل النتائج فى تقليل النيماتودا مع زيادة فى محصول البطاطس وصلت الى ٣٠% مقارنة بالكنترول هي التي احتوت على الاسمدة الحيوية الثلاث بالاضافة الى المبيد الحيوى استانس استنج .