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Comments on the use of biocontrol agents against plant-parasitic nematodes

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Abstract : Plant-parasitic nematodes (PPN) undoubtedly represent a serious threat to the world economy. Growing dissatisfaction with chemical nematicides due to environmental issues obstructing the use of traditional chemicals has created re-directions in the type and choice of applicable nematicides. The role of different beneficial microorganisms ranks high as environmentally friendly biological alternatives to synthetic nematicides. However, in order to maximize the benefit from these biocontrol agents against PPN, a few issues and/or shortcomings in experimentations and applications against PPN have been reviewed herein. It should be highlighted that an evaluation of biocontrol agent efficacy, based on nematode-egg mass index (EI) is better than that based on gall index (GI) because the former index measure nematode fecundity. Moreover, El does not measure nematode reproduction adequately because it does not quantify the eggs produced. Nematode eggs may be a better parameter of sedentary nematode reproduction than GI, EI or other developmental stages. Possible weak links in a nematode's life-cycle that can be targeted for biocontrol by fungal or bacterial antagonists are illustrated herein in more details. Many researchers study the effects of biocontrol agents on their targets of PPN exclusively but the efficacy of these agents on crop yields and/or plant growth parameters as the crux of the matter should also be considered. Moreover, such an efficacy is mainly based on PPN parameters but measuring accumulations and/or activities of pathogenesis-related proteins and other relevant compounds could be used as fast and accurate biochemical markers or components of systemic resistance in plants against PPN infection and reproduction.

Key words: Plant-parasitic nematodes, biocontrol, induced resistance, markers, mode of action.

Introduction

Plant-parasitic nematodes (PPN) are responsible for great losses in crop production systems worldwide. Abd-Elgawad and Askary (2015)[1] reported an average worldwide crop loss of 12.6% which equaled $215.77 billion annual yield loss due to these nematodes for only the top 20 life-sustaining crops based on the 2010-2013 production figures and prices. Moreover, 14.45% or $142.47 billion was an average annual yield loss in the subsequent group of food or export crops. These figures are astonishing, and the authentic figure, when more crops throughout the world are considered, probably exceeds such estimations. On the other hand, numerous relevant and challenging issues have been demonstrating the desperate need of human beings to provide more and better food for an over-populated world. Abd-Elgawad (2014a)[2] stressed the importance of such issues due to reduced number of effective nematicides available and limitation in their use because of environmental and health hazards, renewable manifestation of resistance-breaking nematode pathotypes on many important crops, climate change, increased adoption of intensive agriculture, and potential occurrence of quarantine-nematodes. Therefore, nematode management and research should be more oriented to offer better control of PPN in an environmentally and economically beneficial manner.

In Egypt, as a case in point, such loss estimates due to PPN on 80 crops, 15 of which are ‘life sustaining’, were reported by Abd-Elgawad (2014b)[3] as L.E. 15.85 (= $2.30) billion annually for 2011-2012 production season and prices. Considerable crop losses of vegetables, fruits, and field crops were apparent. For example, such estimates for vegetables only are shown in Table 1. Likewise, numerical estimates of yield losses in other crops such as citrus and legumes are staggering. Therefore, solving PPN problems warrant a lot of more efforts and backup. In this respect, continuous research on different aspects of PPN, especially those factors relevant to crop damage and nematode control, is in progress. As root-knot nematodes (RKN), *Meloidogyne* spp., are among the main important pests causing serious yield losses, such studies are essential for determining their appropriate control strategies. Recently, RKN damage was examined on sugar beet varieties by Youssef *et al*. (2016)[4], on sunflower by Korayem *et al*. (2016)[5] and on cowpea by El-Nagdi and Youssef (2016)[6]. Different management tactics of these nematodes via host-plant resistance, chemical control, biological control, crop rotation and other cultural practices are in progress[7,8]. Despite the importance of any of these tactics, accelerating public concern about overuse of synthetic chemical pesticides has urged researchers to direct a lot of their investigations to biological control agents as safe alternatives to these chemicals with a clear aim at avoiding their health hazards and environmental pollution. Many researchers and stakeholders are addressing such a goal through the development of biocontrol agents as environmentally friendly biological alternatives. For instance, evaluation of soil amended with biological control agents and/or compost for controlling the citrus nematode, *Tylenchulus semipenetrans*, and fusarium dry root rot was carried out by El-Mohamedy *et al*. (2016)[9] on sour orange and by Hammam *et al*. (2016)[10] on Volkamer lime.

Yet, in order to maximize the benefit from this biocontrol approach, I’d like to review and discuss herein some issues and/or shortcomings in experimentations against PPN and offer my point of view towards PPN-management philosophy and applications of biological control agents.

1. Adequate nematode parameters

Extensive research has been conducted on the use of pathogenic or non-pathogenic microorganisms (bacteria, fungi viruses and nematodes) against different species and populations of PPN. For example, McKenry and Anwar (2007)11 found that avirulent M. incognita populations induced systemic resistance against virulent populations of M. arenaria infecting harmony grape rootstocks. Similarly, pre-inoculated avirulent strains of two nematode species (i.e., Meloidogyne incognita and Bursaphelenchus xylophilus) could induce systemic resistance against M. hapla and B. xylophilus in tomato and pine plants as reported by Ogallo and McClure (1995)12 and Kosaka et al. (2001)13, respectively. Different nematode parameters are used for such experimentations. These parameters may include nematode reproduction ratio/factor. A reproduction factor is the outcome of dividing the final nematode population (Pf), by the initial inoculum density of nematodes (Pi). One or more developmental stages of the targeted nematode species are usually considered as Pf.

Table 1. Summary of annual yield losses in vegetables due to damage by plant parasitic nematodes in Egypt* as estimated by Abd-Elgawad [3].

Varieties	Loss (%)	Actual production (metric tons)	Price (L.E./ton)	Actual loss (metric tons)	Loss (Million L.E.)
Tomato	12%	8571050	1500	1168779.5	1753.17
Squash	20%	559598	1000	139899.5	139.9
Green bean	7%	251279	3000	18913.44	56.74
Dry bean	7%	69486	5000	5230.13	26.15
Green cowpea	10%	24277	3000	2697.44	8.09
Dry cowpea	10%	12950	5000	1438.89	7.19
Green pea	12%	180631	4000	24631.5	98.53
Dry pea	12%	124	6000	16.91	0.10
Cabbage	9%	638227	500	63121.35	31.56
Cauliflower	5%	171088	500	9004.63	4.50
Eggplant	20%	1193642	1000	298410.5	298.41
Pepper	22%	650554	2000	183489.59	366.98
Okra	13%	97108	3500	14510.39	50.79
Jew's mallow	6%	80316	1000	5126.55	5.13
Spinach	10%	39413	2500	4379.22	10.95
Mallow	8%	2161	1000	187.91	0.19
Artichokes	10%	387704	1500	43078.22	64.62
Taro	6%	118759	2000	7580.36	15.16
Radish	10%	12000	800	1333.33	1.07
Turnip	8%	32779	500	2850.35	1.43
Lettuce	12%	93661	2000	12771.96	25.54
Carrot	13%	179291	2000	26790.61	53.58
Parsley	8%	88487	1000	7694.52	7.69
Arugula	7%	52281	1000	3935.13	3.94
Egyptian leek	10%	31223	1000	34692.22	34.69
Sweet Potato	7%	319427	750	24042.89	18.03
Strawberry	12%	242297	2000	33040.5	66.08
Beet	10%	3518	2000	390.89	0.78
Pumpkin	17%	1256	1200	257.25	308.7
Watermelon	14%	1874710	1200	305185.35	366.22
Cucumber	15%	587612	1500	103696.24	155.54
Armenian cucumber	15%	50568	1400	8923.76	12.49
Cantaloupe	15%	854204	1500	150741.88	226.11
Melon	15%	89927	1400	15869.47	22.22
Shahad	15%	62716	1600	11067.53	17.71
Potato	8%	4758040	2000	413742.61	827.49
water melon pulp seeds	10%	67274	4500	7474.89	33.64

*Based on 2011-2012 total Egyptian production figures and prices on wholesaling basis; not retail.

For RKN, different nematode parameters have been in use such as nematode gall index (GI), egg mass index (El), eggs, and/or nematode developmental stages within roots and soil for such experimentations. I'd like to stress that an evaluation of biocontrol agent efficacy, based on EI is better than that based on GI because the former index measures nematode fecundity. Moreover, El does not measure nematode reproduction adequately because it does not quantify the eggs produced. Dababat and Sikora (2007)[14] used number of *M. incognita* galls and egg masses per root system in order to assess resistance induced by the mutualistic endophyte, *Fusarium* *oxysporum* strain 162, toward *M. incognita* on tomato. Nematode eggs may be a better parameter of sedentary nematode reproduction than galls and egg masses or other developmental stages. Standardized screening protocols for plant-nematode resistance usually depend on such an accurate measure[15,16] but this application should also be considered while evaluating biocontrol agents against RKN.

2. Modes of action of biocontrol agents vs. weak links in nematodes' life-cycle

Although these modes of action have recently been reviewed[17], further research should reveal more attributes for each of their mechanisms involved in the nematode control. This is especially important for virulence mechanisms of microorganisms such as fungi[18] and bacteria[19] against PPN. For example, the modes of action of nematophagous bacteria may be through: parasitizing, producing toxins, antibiotics, enzymes, competing for nutrients, inducing systemic resistance of plants, and promoting plant health[20]. So, nematophagous bacteria could be grouped based on their modes of action. If so, they can be broadly grouped into parasitic bacteria and non-parasitic rhizobacteria[21]; but they may also split to contain six categories: obligate parasitic bacteria (*Pasteuria* spp.), opportunistic parasitic bacteria, rhizobacteria, cry protein-forming bacteria, endophytic bacteria, and symbiotic bacteria[20,22]. Furthermore, Sikora *et al*. (2007)[23] mentioned three main modes of action employed by bacterial endophytes for the biocontrol of phytonematodes. These are: 1) preventive colonization; 2) immediate antagonism via toxic metabolites; or 3) induced systemic resistance (ISR)[24]. While nematophagous fungi have various virulence mechanisms to manage PPN, predatory and egg-parasitic fungi are the most promising agents due to their readiness for laboratory production, nematode control potential, ability to adapt to different agroecosystems and possible subjection to multitude of studies[18].

Each of these mechanisms affects one or more of the weak links in a nematode's life-cycle. Sikora *et al*. (2007)[23] mentioned nine possible weak links in a nematode's life-cycle that can be targeted for biocontrol by fungal or bacterial antagonists. I'd like to add herein two new links to comply with recent nematological philosophy in terms of using more available research tools for accurate details of nematode data and their interpretations. The new important links are nematode persistence in soil and nematode development (Fig. 1). These weak links could generally be explained by Dababat and Sikora (2007)[14] who found that inoculation of tomato plants with the non-pathogenic fungal endophyte *Fusarium oxysporum* strain 162 resulted in a significant reduction of *Meloidogyne incognita* infection; due in part to induced resistance after fungal inoculation. For clarification of the two new links, suppressiveness of soils, for instance, to *Meloidogyne arenaria* race 1 in a peanut field located near Williston, Levy County, Florida, USA, was observed by Dickson *et al*. (1994)[25] over a period of several years of crop production; indicating poor nematode persistence in soil. Also, Orion *et al*. (1980)[26] indicated that higher concentration of ammonia released during the decomposition, by bacteria, of organic additives/amendments could inhibit the complete development of syncytium which is necessary for the nematode development, since syncytium is the food supplier for the root-knot nematode. Moreover, in order to bring PPN populations below a threshold level, it is also important to note that these biocontrol agents may have a determined specificity against definite stages or species of the nematodes. Eventually, determining which stage in the life-cycle of a nematode species/strain is the best target for biocontrol tactics necessitates carefully conceived and well carried out experimentation.

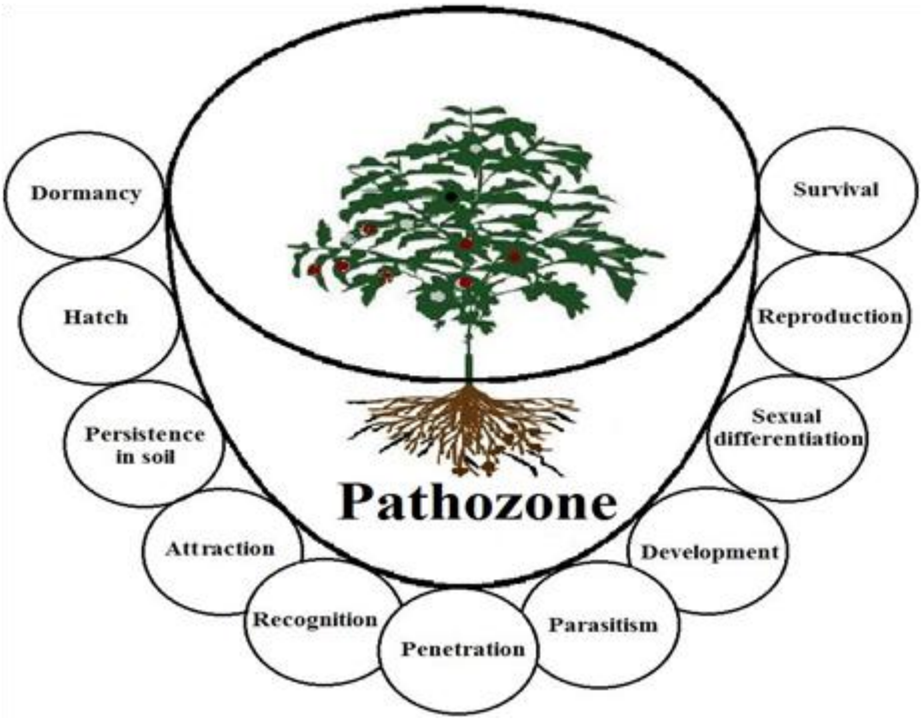

Fig. 1. The pathozone of soil surrounding the root system of young plants, and eleven possible weak links in a nematode's life-cycle that can be targeted for biological control by fungal or bacterial antagonists, modified from Sikora *et al*., [23].

3. Effects of biocontrol agents on plant growth parameters

Although investigating the effects of biocontrol agents on their targets of PPN are usually the direct objectives of nematode management studies, we should consider the efficacy of these agents on crop yields and/or plant growth parameters as the crux of the matter. In other words, the former effects are of course desirable. Valid as they are, the relationships of these agents with their surroundings are frequently complicated by many other factors that can favor or disfavor such effects on PPN and plant growth parameters. To name but few of these factors are the carry-over stress effects from one crop/year to the next, cyclic nematode population growth, the interaction between pest and plant stresses and the capacity for vigorous trees/plants to support (tolerate) larger populations than those with a limited number, of severely infected roots and consequently disorganized vascular system.

Since biocontrol agents have been gaining more consideration as novel, safe and potential tools to provide major benefits for PPN management, their consistent and skillful performance should be addressed. Therefore, they should fit with a range of complex intrinsic (microbial), external (host and environmental) and most importantly, integrated factors. These factors make up the specific context in which biocontrol agents are used[27]. Accommodating each of these factors is an essential step towards enhancing the level and reliability of their activity not only against PPN but also to attain crop yield increase. If properly handled, these factors can also act synergistically with such agents to increase consistency and efficacy. Rational management decisions can be made only by analyzing the interactions naturally occurring among host plant-nematode target-soil–microbial control agent-environment; a five-party interaction (Dong and Zhang, 2006)[28]. Therefore, nematode population levels may affect fruit yields differently under various conditions[29]. Thus, the most sustainable method to PPN control should integrate several tools and tactics via integrated pest management (IPM). With better and sound tackling of these interactions, biocontrol of nematodes should be more fine-tuned via strain selection and improvement (e.g. for compatibility with agrochemicals and interaction with other biotic and abiotic factors), mass production, formulation, packaging, successful wide-scale testing and field application of biocontrol agents. Efficient quality control and standardization, registration, and sound market assessment (product efficacy, cost, profit margins, shelf-life, ease-of-use, market acceptance, product coverage and stability) should be pursued to contribute to both development of efficacious biocontrol for PPN and yield increase. These issues and technical advances in their production and utilization will provide further opportunities to integrate the use of biocontrol agents in management programs of nematode pests.

4. Induced plant resistance

Systemic resistance can be induced via different approaches. Defense mechanisms in induced plants can be directly activated after infection by pathogens including fungi[30], bacteria[31], and nematodes[12] or after treatments by chemicals such as salicylic acid, benzothiadiazole and 2,6-dichloroisonicotinic acid[32], or following challenge of the induced tissue by parasites[33]. The latter case is known as priming which occurs in systemic acquired resistance (SAR), induced systemic resistance (ISR), and mycorrhiza-induced resistance (MIR). The molecular mechanisms of priming are based on epigenetic modifications that suppress or enhance the transcription of key regulators of the immune system. Changes in methylation and acetylation status of DNA and histones have been associated with the activation of immune related genes in plants. Therefore, a combination of epigenetic control mechanisms and an abundance of signals are probably to be at the base of long-lasting immune memory in plants[33,34]. For example, the addition of the nonpathogenic endophytic *Fusarium oxysporum* strain 162 to the inducer side of the split-root system of tomato plants resulted in a significant reduction of *Meloidogyne incognita* penetration in the responder side of the split-root system, and in reduced production of galls and egg masses, 2 and 5 weeks after nematode inoculation, respectively[14]. Consequently, the authors[14] concluded that induced resistance was an important component of the overall mode of action of non-pathogenic endophytic *F. oxysporum* on tomato plants and a factor that influences plant based changes in nematode behavior. Interestingly, induction of resistance against many pathogens has frequently been correlated with the synthesis and accumulation of salicylic acid, jasmonic acid, pathogenesis-related proteins, and enzymes such as catalase and peroxidase in different plant species[33,35]. Yet, some references have not considered such correlations. Therefore, I'd like to emphasize the need to measure also accumulations and/or activities of these compounds as fast and accurate biochemical markers or components of systemic resistance in plants rendering them less suitable for PPN infection and reproduction.

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