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REVIEW ARTICLE

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Nematode spatial distribution in the service of biological pest control

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Abstract

Sound nematode sampling techniques and related measures can gain biocontrol merits from determining their spatial (geographic and temporal) distribution patterns. These latter may be compared to other relevant biotic/abiotic forces to unfold complexities of ecosystem dynamics into quantifiable variables for better development of controlling plant pests/pathogens. Because biotic/abiotic factors are mostly more sensitive in biocontrol tactics than chemical pesticides, it becomes increasingly important to quantify such variables. Herein, pros and cons of common models used to characterize such patterns are reviewed. Adjusting models to acquire more accurate and targeted outputs for cost-effective and reliable plant-parasitic nematode management are presented, e.g., revising optimum sample size. Single models can act for the nematode-count transformation to meet assumptions necessary for parametric statistical analyses and consequently attain valid and accurate treatment comparisons. Yet, it is preferable to use more than one model to demonstrate more aspects of nematode distributions and optimize pest control in integrated pest management (IPM) plans. Harnessing these aspects will enable best seed-location matching, leverage variable rates of the used bionematicides and grasp relationships between beneficial/harmful organisms in space and time for alert IPM. Entomopathogenic nematode spatial (horizontal/vertical) distributions can mirror shifting in their key community dynamics such as parasitism and competition. To overcome limitations related to these models, incorporating emerging innovations like the PCR-based approaches to identify and quantify species (e.g., qPCR versus high-throughput sequencing), bioinformatics and volatile organic compounds as signals for soil inhabitants are discussed.

Keywords Agricultural sustainability, Bioinformatics, Index of dispersion, PCR-based methods

Background

The rapid increase in global population without similar elevation in agricultural production necessitates optimal exploitation of all factors that enhance such a production. The ongoing progress in the biological control realm of plant pests and pathogens should optimize techniques applied for nematode spatial distribution patterns to alleviate this issue. Two major nematode groups are practically engaged in this realm where such techniques should be harnessed for both managing pests/pathogens and boosting durable agriculture. Plant-parasitic (PPNs) and entomopathogenic nematodes (EPNs) represent important groups in various systems of crop production worldwide. Optimizing methods for characterizing their spatial distribution patterns can benefit integrated pest management (IPM) via utilizing EPNs as a biocontrol agent (BCAs) and controlling PPNs as pests (Abd-Elgawad 2021). While EPNs can play a significant role in biological control for sustainable food production (Koppenhöfer et al. 2020), global staggering figures of crop losses caused by PPNs were routinely detailed.

Various facets of nematode biology and ecology are basic to optimize PPN management and EPN biocontrol strategies. Hence, substantial challenges are faced in grasping where their multi-trophic interactions uncover and how to identify and decipher them. Basically, in

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order to unfold the complexities of their ecosystems and biological dynamics into quantifiable variables, three fundamental models of all possible relationships between the arithmetic mean (μ) of nematode counts in a population and their variance (O^2) were demonstrated. These are the positive binomial ($O^2 < \mu$), the Poisson series ($O^2 = \mu$), and the negative binomial ($O^2 > \mu$). The three models directly represent probability distributions. Therefore, converting each model to a frequency distribution is obtained by multiplying the nematode-sample size in such a probability. Obviously, the obtained frequency distribution can serve the following aspects: interpret the spatial dispersion of the nematode population in mathematical terms (ecological meaning), calculate errors of nematode-population parameters, compare spatial (horizontal or vertical) changes in nematode population levels, and estimate the effects of natural/introduced ecological factors (Salama and Abd-Elgawad 2010). In this context, the root-knot nematode (RKN) *Meloidogyne hapla* Chitwood (Tylenchida: Heteroderidae) populations manifested aggregated distribution because their dispersal fit the negative binomial (Wheeler et al. 1994). On the other hand, two populations of the root lesion nematode (*Pratylenchus penetrans* Stekhoven [Tylenchida: Pratylenchidae]) showed random distribution in soil as their dispersal fit Poisson distribution (Wheeler et al. 1994). The third model, positive binomial, is usually not of proper usage in field-nematode distribution because it fits only evenly distributed microorganisms. However, this model is practical for fixing probability levels for checking a nematode-infested unit in any sample/lot sizes with various infestation intensities during agricultural quarantine programs.

The present review addresses developments in nematode distribution patterns to demonstrate their economic contributions to current management schemes. Yet, as biocontrol tactics are often more responsive to biotic and abiotic factors than chemical pesticides, there is increased importance to address these patterns for the biological control realm. They can assist in both safe and effective management of PPNs and biocontrol applications of EPNs. The review presents pros and cons of common models used in the near past and currently. Revision of models for more cost-effective, value-added approach for pest management, especially for the decision-maker is discussed. Also, adjusting nematode distribution models to derive more adequate and precise/accurate conclusions for biocontrol of PPNs is presented. To overcome or circumvent limitations linked to current nematode distribution-related models, emerging innovations are suggested. These include the PCR-based techniques to identify and quantify species (PCR, qPCR, and high-throughput sequencing), bioinformatics, and volatile organic compounds as cues for not only nematodes but also related soil inhabitants and attacked plants.

Pros and cons of common models for practical nematode distribution patterns

Having stated the above-mentioned basic models of distribution patterns, developed models that have been applied to the distribution of nematodes should be addressed. These models are applied to substantially assist in unfolding the complicated ecosystem dynamics into definite values that represent the indices of dispersion for the studied nematode populations. A few models may further be developed for multifaceted objectives.

A definite model may have certain advantages over others. For example, Taylor's power law (TPL) established by Taylor (1961) is used not only to define nematode index of dispersion but also to develop nematode-optimum sample size as well as to apply nematode-data transformation to validate terms required for parametric statistical analyses (Ghaderi et al. 2012). Because TPL was not originally applied in nematology (Taylor 1970), nematologists (McSorley et al. 1985) have modulated this law in the following form: the statistical variance (S^2) of a nematode population is proportional to a fractional power (b) of the arithmetic mean of nematode counts (X): $S^2 = aX^b$ or $\log S^2 = \log a + b \log X$, where: a and b are nematode population parameters, a is contingent on the sample size and b represents the index of nematode dispersion. Therefore, knowing the values of a and b in TPL, the sample size N^* (n or N) could be computed from: $N^* = (t_{\alpha[n-1]}/D)^2\ a\ (\overline{x})^{b-2}$ or $N = (1/E)^2\ a\ (\overline{x})^{b-2}$, as N^* or N is the sample number, $t_{\alpha[n-1]}$ is the proper Student's t estimate for confidence profiles of $1-\alpha$ and $n-1$ degrees of freedom, and sampling reliability or accuracy is determined either with reference to the standard error to mean ratio (E) or the fraction resulting of dividing the half-width of the confidence limit on the arithmetic mean (D) of the samples (Ferris et al. 1990). Much informative data on applying TPL could supply key conclusions of the studied nematodes. At 1000 *Tylenchulus semipenetrans* Cobb (Tylenchida: Tylenchulidae)-juveniles and males/100 cm^3 soil (0.20 standard error to mean ratio), sample sizes were determined from TPL to be 12 trees in the geographic survey at Florida (USA) and 11 trees in the temporal (Duncan et al. 1989). Deletion of data concerning the small-patches formed by the citrus nematode *T. semipenetrans* populations in the surveyed citrus orchards from the geographic survey led to a 17% decrease in the theoretical sample size. On the contrary, they assessed such a sample size in scattered *T. semipenetrans*-infested groves as 69 trees. Furthermore, they could discriminate the group of citrus orchards with smaller patches of *T. semipenetrans*-infected trees from that from

large-patch orchards based on the significant difference ($P < 0.05$) between their related slope values of the law. This information is valuable for assessing the feasibility of site-specific bionematicide usage and other control measures for IPM in the surveyed citrus orchards. They found that a data transformation using $x^{0.23}$ derived from TPL for comparing arithmetic means in experimental treatments could minimize ($P \leq 0.01$) variance ratios compared to untransformed data. In favor of TPL, $x^{0.23}$ transformation enabled separation of treatment population levels in one experiment where statistical difference was insignificant as log-transformed data were applied (Duncan et al. 1989). Rumiani et al. (2021) designated *T. semipenetrans*-sampling patterns in two sets of actual and virtual compositions using TPL to describe the nematode spatial distribution and derive optimum sampling plan. Consequently, they recommended two composite samples from the inner (four cores) and outer (six cores) areas of the watering dripline in order to attain cost-effective and reliable approach via the best sampling scheme of the nematodes. These techniques that induce favorable outcomes such as inexpensive sampling plans and acceptable reliability range should serve emerging biocontrol and IPM strategies.

Clearly, assessing crop losses caused by pests and pathogens should also consider all yield-forming and -reducing elements in order to boost crop yield and durability evaluation. Hence, the importance of revising and/or adjusting these models whenever possible is apparent. In this trend, an iteration procedure for more accurate assessment of nematode-optimum sample size may be practical. In consequence, the optimum number of the root-knot nematode (*Meloidogyne incognita* Kofoid & White [Tylenchida: Heteroderidae]) samples, needed to achieve a definite level of accuracy/reliability was 402 and 389 samples before (McSorley et al. 1985) and after (Abd-Elgawad 2023) the fine-tuning procedure, respectively (Table 1). Furthermore, the TPL may be arranged differently to find out various levels of sampling accuracy and precision. Because cost, time, and effort restrict the precision/accuracy of sample size, a decision maker has to reconcile via cost and benefit tradeoff. This is usually materialized through accepting a tolerable level of accuracy/precision given several of such levels. For each of these levels, TPL was applied to assess cost of its corresponding samples. Collectively, for enlightening the decision-maker via various options, TPL was re-set to solve for sampling accuracy/precision levels connected to fixed, cost-determined, sample sizes as a way forward in crop safeguard and pest control. Admittedly, if these costs to assess the PPN population in the field are more than to apply any of the possible BCA or other control alternatives, the best decision is to directly apply the pest management as insurance without counting the PPN population. Another benchmark is to consider confidence limit in such statistical handlings. It should mostly be well-below or above the economic threshold levels. As identification of novel nematode species and strains is

Table 1 Optimum nematode sample size needed to achieve a 25% level of reliability as defined in terms of standard error to mean ratio (*E*) or confidence interval half-width to mean ratio (*D*) with iteration

Mean count per sample	Number of samples via *E*	Student's *t* value	Number of samples via *D*	References
Criconemella spp.: The power law parameters $a = 3.076$, $b = 1.218$				
10	8	2 (assumed)	33	Abd-Elgawad and Hasabo (1995)
		2.037 ($n = 33$)	34	
		2.035 ($n = 34$)	34	
100	1	2 (assumed)	5	
		2.776 ($n = 5$)	10	
		2.262 ($n = 10$)	7	
		2.447 ($n = 7$)	8	
		2.365 ($n = 8$)	8	
Meloidogyne incognita: The power law parameters $a = 4.77$, $b = 2.12$				
10	101	2 (assumed)	402	McSorley et al. (1985)
		1.966 ($n = 402$)	389	
		1.966 ($n = 389$)	389	
5	93	2 (assumed)	370	
		1.966 ($n = 370$)	358	
		1.967 ($n = 358$)	358	

The *t* value is either assumed as 2 for 95% confidence interval or iterated using its tabulated value from: http://www.danielsoper.com/statcalc3/calc.aspx?id=10 (Abd-Elgawad 2023)

quite progressing using deep learning patterns for their automatic identification (Shao et al. 2023), these confidence intervals should be checked.

The frequently aggregated spatial distributions of many insect pests (Southwood 1978) and their BCAs such as EPNs (Stuart et al. 2015) have been documented. Unless nematode counts are transformed via an index of their dispersion, such distributions pose problems to apply parametric statistical analyses especially those closely related to nematode experimentations, e.g., *t*-tests, analysis of variance (ANOVA), and regression tests. They are usually imperative to sound conclusions as they help in fixing the design needed for both biocontrol assays and field experiments as well as test hypotheses. For instance, indices of dispersion for both the red palm weevil, *Rhynchophorus ferrugineus* Olivier (Coleoptera: Curculionidae) and the EPN *Heterorhabditis bacteriophora* Poinar (Rhabditida: Heterorhabditidae) were basically determined given their clumped distributions (Salama and Abd-Elgawad 2010). The authors used TPL to define the distribution patterns of *R. ferrugineus* larvae, pupae and/or adults, and the index of dispersion (k) for the negative binomial for the patterns of *H. bacteriophora*. Consequently, optimum sample size for both the pest and its biocontrol agent was determined. The EPNs were uniformly applied to the orchard soil, but they showed contagious distribution immediately and 2 weeks after application and even distribution after 4 weeks. Interestingly, Wilson et al. (2003) concluded that EPN introduced evenly in soil was more effective for controlling white grubs (larvae of scarab beetles) in turfgrass farm than EPN applied in patches. Hence, as both *R. ferrugineus* and *H. bacteriophora* manifested mostly clumped distribution, two principal scenarios for bio-controlling the insect via both augmentation technique of the introduced EPNs or taking advantage of endemic EPNs. In the first scenario, the inundative release of *H. bacteriophora* for immediate control of the weevil may be through applying EPN patches to the weevil/pest clumps to achieve decisive outputs provided that edaphic and biotic factors are adequate for *H. bacteriophora* biocontrol activity. Otherwise, it is quite possible that nematodes applied evenly can clump to match the aggregations of their target insect hosts. In this context, EPNs had fulfilled high control levels of citrus root weevil, *Diaprepes abbreviatus* Linnaeus (Coleoptera: Curculionidae), in citrus orchards of Florida (USA) before combating the bacterial disease huanglongbing via new citriculture regime which negatively affected EPNs. For endemic EPNs, the uneven and low levels of EPN-infected *R. ferrugineus* would mostly lead to low suppression levels of the pest unless indigenous EPNs with superior biocontrol attributes are found in the second scenario. Shehata et al. (2020) discussed such key attributes, e.g., EPN foraging behavior, virulence, persistence, and reproductive potential; that can possess marked impact on EPN biocontrol performance.

Other techniques based on common models to assess distributions of many microorganisms including PPN and EPN species have been used (Gorny et al. 2020). In addition to the above-mentioned ones, these models include grading and mapping spatial autocorrelation (Campbell and Noe 1985), evolving multi-dimensional maps to specify spatial patterns of fairly immobile organisms, and applying spatial analysis by distance indices (SADIE) erected and developed by Perry (1998) to initially define distribution patterns of insect pests in mathematical terms; later applied on various nematode groups especially EPN (Spiridonov et al. 2007) and PPN (Kabir et al. 2018) populations. They aimed not only to achieve the aforementioned objectives but also to develop/evaluate further control measures against pests and pathogens. To optimize the output of these models, they should consider all factors dictating the distributional behavior of any studied nematode population through time/space. These factors may be defined by inherent attributes of the individuals and their conciliation with ecological elements, interacting biotic forces, and food resources as well as production practices such as nematicidal application, soil tillage and irrigation (Ferris et al. 1990). Moreover, the potential for considerable spatial variability and consequently much time, efforts, and costs are also required to assemble, transfer, and examine nematode samples to identify/quantify their populations. Therefore, Gorny et al. (2020) stated that presumptions are often necessary concerning nematode spatial distributions, lags, and density parameters of their populations. These presumptions are sometimes solely based on considerable empirical evidence. In this regard, TYL was established as a rule of thumb. Because the mechanism of TPL relies on solely applying linear regression on practiced and empirical data rather than theory, it is still debated among relevant researchers. Nevertheless, TPL applications proved to be adaptable to various taxonomic orders of numerous organisms and utilized to investigate their natural indices of dispersion, a tome has been published to materialize this fact (Taylor 2019). For example, the exponents of TPL fluctuated systematically to coincide with potential biotic/abiotic drivers of distribution patterns in both space and time of fishes in the North Sea which could offer ecological assets of the examined settings (Cobain et al. 2019).

On the contrary, SADIE is praised because it distinctively applies spatial and temporal statistics in an approach that also supplies actual profiles to inferences concerning spatial masses and the spatial/temporal links between variables. It is also a realistic measure backed by

sound theories. To smooth and expedite its usage, SADIE software package comprising its major indices and graphical manifestations were composed as complimentary programs (Winder et al. 2019). Abd-Elgawad (2023) compared its index of aggregation (I_a) values for four EPN distribution patterns in various localities. The index values varied according to the form of the measured EPNs which originally function for biological control against various pests and pathogens. These forms included distributions of EPN-infected *Galleria mellonella* Linnaeus (Lepidoptera: Pyralidae) larvae over time (Wilson et al. 2003), EPN-infective juveniles (IJs) related to one of 4 categories of different physiological ages (Spiridonov et al. 2007), IJs of both *Heterorhabditis bacteriophora* and *Steinernema carpocapsae* Weiser (Rhabditida: Steinernematidae) extracted from *G. mellonella* larval baits introduced several times (Bal et al. 2017), and EPN-infected *G. mellonella* larvae by natural *Heterorhabditis indica* Poinar, Karunakar & David (Rhabditida: Heterorhabditidae) populations in an orchard cultivated with mixed mango and citrus trees. Because EPNs mostly manifest aggregated distributions, Stuart and Gaugler (1994) stated that these distribution patterns can reflect key performance of the natural polyspecific nematode population densities by altering the dynamics of parasitism, competition, and predation. For such trends, SADIE could be combined with other approaches to investigate soil food webs in citrus orchards for developing effective and safe biocontrol methods. Moreover, a complementary model (Li et al. 2012) may be necessary to optimize setting of spatial heterogeneity in definite positions as SADIE cannot tackle it properly. Such a model is beneficial as clusters of the examined organisms are situated on elongated/square domains and near the edges of the surveyed areas. In such positions, additional method can discriminate clusters with small radius (smaller sample size) than that of SADIE. Clearly, geostatistical models provide valuable parameters that can plot nematode counts in samples to their related locations to illustrate distribution patterns and determine definite values at un-sampled locations. Geostatistical equations can reliably employ global positioning systems (GPS) to fix the sample location (Goovaerts 1999). On the negative side and contrary to SADIE (Perry 1998), this set of equations does not employ tests to evaluate any probability level for statistical significance of the inferred patterns. SADIE can determine such a level of spatial relations between different organisms or the same organism over times (Perry 1995). Increased attention is being given to SADIE, but it is still less used in nematology than TYL.

Eventually, these models with their indices should preferably be applied in a complementary approach rather than applying single model as in a few of previously published papers. Obviously, a complementary technique can demonstrate more characteristics of their spatial distribution patterns. Practically, two indices were simultaneously used (SADIE and semivariogram modeling) to boost proper sampling procedures and to determine definite sites for controlling *Meloidogyne hapla* and *Pratylenchus penetrans* in commercial potato fields to save unnecessary costs in crop protection (Gorny et al. 2020). Additionally, contrary to samples obtained near to each other, samples gathered far apart are more impacted by various microhabitats. Hence, using SADIE analyses and semi-variogram together is more depictive of distribution patterns to better grasp the spatiotemporal dynamics of EPNs (Stuart et al. 2015) and PPNs (Gorny et al. 2020). Definite technologies and statistics for spatial/temporal distributions were also drawn to offer useful devices that can analyze point distributions. They can enhance discovery and characterization of spatial heterogeneity in terms of clustering or gradient patterns (Perry et al. 2002). Moreover, analysis of these patterns may also attest novel information about significant links among pathogen taxa, e.g., affinity/co-occurrence (Pethybridge and Turechek 2003) or effects of certain forces such as edaphic factors on spatial pattern, crop damage, and multiple interactions among the examined taxa.

Incorporation of advanced technologies into nematode distribution patterns to boost biological pest control

A novel era that includes sophisticated technologies is emerging. These technologies should imply the distribution patterns to improve biocontrol of pests. As such technologies are being explored, experimented, or applied; suffice it to point to some examples distinctly expressing their counterparts.

Use of the PCR-based approaches to identify and quantify species

Detecting new EPN species and strains is hugely ongoing via sound and swift morphological, biochemical and molecular devices (Bhat et al. 2023). Evaluation of their community structure and distribution patterns in various ecological zones can assist disclose their biocontrol potential against key crop pests (Dritsoulas et al. 2020). As EPNs are broadly and naturally distributed in soils across most continents, their detection from samples comprised several methods; baiting techniques, binocular vision, and use of polymerase chain reaction (PCR)-based approaches. All the methods can EPN biocontrol tactics that use classical, augmentation/inundative and conservation biocontrol approaches to induce acceptable pest mortality rates. Although the inundative biocontrol is the mostly used technique, its released

EPN populations often decrease to low levels within few post-application weeks. To address this issue, Duncan et al. (2007) developed an assay to identify/quantify EPN-microbial predators immediately isolated from EPNs extracted from soil. However, this assay needs an incubation interval that might bias their population estimates. Additionally, EPN assessed indirectly via baiting techniques are imprecise because infection rates of the baits are both species specific and relied on edaphic factors, e.g., soil temperature, moisture, and porosity. While baiting techniques are time consuming and labor intensive, binocular identification of EPNs extracted from soil requires further taxonomic expertise. Instead, these authors proposed that EPNs and their linked parasites/predators should be quantified immediately after their extraction from soil based on the molecular data of EPN taxonomy (Nguyen and Hunt 2007). For instance, phylogenetic research on the D2D3 and ITS regions of EPNs with extensive diversity in Florida groves manifested the additional occurrence of species-complex in the *Steinernema glaseri* (Steiner) Wouts, Mráček, Gerdin & Bedding (Rhabditida: Steinernematidae)-group in broadly separated groves. Using real-time quantitative PCR (qPCR) assays, Campos-Herrera and Lacey (2018) reported that six naturally distributed species of EPNs could be detected and quantified; *Steinernema riobrave* Cabanillas, Poinar & Raulston (Rhabditida: Steinernematidae), *Steinernema diaprepesi* Nguyen & Duncan (Rhabditida: Steinernematidae), *H. indica*, *Heterorhabditis floridensis* Nguyen, Gozel, Koppenhöfer & Adams (Rhabditida: Heterorhabditidae), *Heterorhabditis zealandica* Poinar (Rhabditida: Heterorhabditidae), and an undescribed species in the *S. glaseri* group in addition to an exotic species (*S. glaseri*). They could adequately design and use species-specific primers and TaqMan® probes related to the ITS rDNA region. Consequently, they developed a related protocol that can detect even an individual EPN added to a nematode population. Such a protocol is really dedicated to serve biocontrol realm especially for EPNs. This technique could not only detect/quantify soil-inhabiting organisms such as EPNs and their related competitors of free-living nematodes as well as ectoparasitic bacteria and nematophagous fungi in the targeted soil but also investigate the EPN soil food web in the studied fields (Campos-Herrera and Lacey 2018). As a merit of this molecular approach in biocontrol tactics and strategies, Campos-Herrera et al. (2016) used qPCR to unveil sympatric distributions of EPN species and disclose their low levels in samples. On the contrary, the insect baiting technique couldn't.

Furthermore, the emerging high-throughput sequencing (HTS) technique has shown greater potential to characterize EPN communities and their natural enemies in soil than qPCR. The HTS was not only as efficient as qPCR in detecting all existing EPN species but also could discover more species of EPNs, as BCAs, than did qPCR (Dritsoulas et al. 2020). Therefore, further designed boosted HTS-related primers to distinguish EPN species along with previously applied primers of D2–D3 region were set for detection of the co-occurrence of EPN-natural enemies of microarthropods in soil (Dritsoulas et al. 2021). They aimed also at characterizing EPN biogeography for perfection of augmentation and conservation biocontrol strategies given the used fine-scale taxonomic resolution of HTS. This resolution could separate firmly related species of EPNs that have divergent key attributes, e.g., host specificity and certain habitats. Interestingly for the Egyptian biocontrol tactics in Egypt, the HTS detected dominant and consistent *H. indica* population levels in El-Beheira governorate that was inversely proportional to species' plenty of the nematophagous mites in the family Rhodacaridae (Dritsoulas et al. 2021). Also, qPCR that characterize spatial distribution of natural communities, relationships between total EPN species and *Fusarium solani* (Mart.) Sacc. (Hypocreales: Nectriaceae) clusters (high density) and gaps (low density) in terms of their spatial patterns in a citrus orchard surveyed over different times could be illustrated (Wu et al. 2019). Ultimately, to enhance the biocontrol potential of such EPNs, dual-purpose, sequential, and co-application of EPNs with extra compatible production inputs may be conducted.

Utilizing bioinformatics

Studies on the significance of biological pest control in the realm of nematology are getting a swift pace motivated by progresses in bioinformatics. Bioinformatics devices can map the interactions of the active ingredients in biopesticides with their biological objects and tackle multifactorial diseases (Gupta et al. 2023). Important contributions to this realm via computational devices/in silico methodologies were recently reviewed. For instance, O'Halloran (2001) erected a computer soft package named CRISPR-PN2 as a resilient and genome-aware platform to assist in genetic assays on parasitic nematodes. It supplies flexible use and control over the automated sketch of specific pilot RNA sequences for CRISPR experiments on PPNs. This computer program assists in enabling high-throughput gene editing at the given scale. Clearly, such an editing may help in boosting desired traits in plants that do not possess enough resistance sources against PPNs. Furthermore, Ibrahim et al. (2019) discussed such an editing to overexpress the genes accountable for the biological control process of *Paecilomyces javanicus* (Friedrichs & Bally) Br. & Sm. (Eurotiales: Thermoascaceae) to activate plant protection and/or

enforce the plant immunity against infection with *Meloidogyne* spp. On the other hand, computing both HTS and qPCR data to fit TPL (Dritsoulas et al. 2020) resulted in inferring that sample accuracy was significantly better using HTS ($r^2=0.95$, $P<0.01$) than qPCR ($r^2=0.76$, $P<0.01$). Moreover, as abiotic/biotic factors can impact EPN biocontrol potential, Wu et al. (2019) ran SADIE soft program and found significant spatial links between the fungus *F. solani* and polyspecific EPN communities (*S. diaprepesi*, *H. indica*, and *H. zealandica*) during a 6-month-citrus grove survey. While the EPNs showed clumped distributions during three continuous winter months, the fungus clumped during the previous month, but such a sketch was not found during the spring. The *F. solani* clumps in the surveyed plots was also linked to that of total nematodes the previous month with one-month time lag ($r=0.17$, $P=0.02$). The authors assumed that such a pattern may be at least partly due to the effect of soil moisture on both EPNs and *F. solani*. These quantitative relationships between *F. solani* and EPNs depicted a field mutualistic relationship between both organisms. Wu et al. (2019) concluded that such relationships may have had measurable impact on the spatial pattern of EPNs and *F. solani* at the landscape scale sketched in the citrus orchard.

Volatile organic compounds as cues to strengthen biocontrol strategies

Numerous organisms can produce volatile organic compounds (VOCs) with olfactory signals that can play crucial role among various organisms to communicate and react to their settings. Growing interests in materializing the full biological control spectrum toward safe and effective pest management have motivated researchers to study and exploit these VOCs. Silva et al. (2019) found that VOCs emitted from dry macerates of certain plant species could reduce *M. incognita*-second-stage juveniles (J_2) motility to about 0% and minimize egg hatching by 47% compared to the untreated check. They recorded that the water exposed to VOCs from *Cymbopogon nardus* (Linnaeus) Rendle (Poales: Poaceae), *Piper nigrum* Linnaeus (Piperales: Piperaceae), and *Bertholletia excelsa* Humb. & Bonpl. (Ericales: Lecythidaceae) minimized the motility of the J_2 by 42%, whereas *Brassica oleracea* Linnaeus (Brassicales: Brassicaceae) achieved almost full immobility. Furthermore, VOCs from *B. excelsa* and *B. oleracea* shoots killed J_2 and minimized the number of galls and eggs in *M. incognita*-infected tomato roots. Purified 3-pentanol and dimethyl disulfide (DMDS) extracted from *B. oleracea* volatilome (Silva et al. 2018) inhibited egg hatching by 88.4% at 918 mg/l and by 96.8% at 176 mg/l, respectively. All their tested species generated VOCs toxic to J_2 and eggs. Since *M. incognita*-J_2 and eggs are key life stages in terms of RKN management, these VOCs should be of practical use to decrease RKN populations in soils prior to host infection or to prime new nematicidal products.

Rasmann et al. (2005) reported that roots of the most European maize lines release the insect-induced belowground VOC (E)-β-caryophyllene that can robustly attract EPNs. The roots release, it as a reaction to feeding by *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae) larvae, a key pest in Europe and North America. They recorded a fivefold increase in EPN infection rate of the pest larvae on a (E)-β-caryophyllene-producing maize variety than on a non-producing variety used in North America. Adding (E)-β-caryophyllene to the soil close to the latter variety minimized the emergence of adult *D. v. virgifera* sharply. Rasmann et al. (2005) concluded that extending the geographic distribution of (E)-β-caryophyllene-producing maize variety to North America or developing novel varieties there that can free the VOC in proper amounts should help boost the biocontrol effectiveness of EPNs against root pests such as *D. v. virgifera*.

Two VOCs, 1-pentanol and 1-octen-3-ol (octenol), released by *F. solani* can attract the EPN *S. diaprepesi* (Wu and Duncan 2020). Octenol can attract the fungivorous fruit fly larvae (*Drosophila melanogaster* Meigen [Diptera: Drosophilidae]), but repel the non-fungivorous citrus root weevil larvae (*D. abbreviatus*). With even distribution of both insects in soil, these VOCs hindered the infection of *D. abbreviatus* larvae by *S. diaprepesi* but eased *S. diaprepesi* infection of *D. melanogaster* larvae. Therefore, Wu and Duncan (2020) assumed that octenol could bring both the flies and EPNs to same sites, but provoked greater separation of EPNs and root weevils than happened in the absence of octenol. They proposed that EPNs may have developed to harness these VOCs for bringing fungivorous insects, whereas non-fungivorous insects may not.

Conclusions

For reliable and perfect biocontrol of plant pests and pathogens, a full spectrum of management strategies engaging various techniques is needed. The intimate disease triangle percept—that crop loss happens exclusively as a host and pest/pathogen co-occur in a proper setting—mirrors a side focused to currently directed trends for expanding the usage of sampling both nematode communities and their associated microorganisms. Because biotic and abiotic factors are often more responsive to biological pest control tactics than chemical pesticides, it is quite important to quantify such elements. Therefore, when commercial potato fields in New York, USA, were challenged with extensive crop losses caused

by several key PPN species, researchers relied on spatially straightforward knowledge of PPN populations in order to back the evolvement of targeted, site-specific pest control measure, minimize overall field inputs, and avoid environmental pollution. Such knowledge of nematode spatial distribution patterns can be inferred from mathematical models to quantify biotic and abiotic factors interacting with the nematodes. Positives and negatives of such models related to the nematode patterns are reviewed. Although some researchers tend to apply only one model in their studies, it is preferable to use more than a model in order to illustrate more facets of nematode distributions and optimize pest management programs. Exploiting such facets will enable best seedlings-zone matching, leverage variably used rates of the bionematicides, and understand relationships among biotic and abiotic forces in space/time for sound pest control strategies. Entomopathogenic nematode spatial (horizontal/vertical) distributions can also mirror altering in their key community dynamics such as parasitism, predation, and competition. To overcome negatives relevant to these models, incorporating emerging technologies such as the PCR-based methods, bioinformatics, and olfactory cues by volatile organic compounds that can react with the nematodes, other pests, and/or natural enemies are discussed.

Abbreviations

BCAs	Biocontrol agents
GPS	Global positioning systems
IJs	Infective juveniles
IPM	Integrated pest management
J_2	Second-stage juveniles
PCR	Polymerase chain reaction
PPNs and EPNs	Plant-parasitic and entomopathogenic nematodes
qPCR	Real-time quantitative PCR
SADIE	Spatial analysis by distance indices
TPL	Taylor's power law
VOCs	Volatile organic compounds

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MMA suggested the idea, collected the literature necessary for the manuscript, wrote and reviewed the manuscript. Also, the author read and approved the manuscript.

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Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

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