Accuracy and precision of phytonematode sampling plans

Mahfouz M. M. Abd-Elgawad

(*Phytopathology Department, National Research Centre, EI-Tahrir St., Dokki 12622, Giza, Egypt*)

Abstract: Sampling accuracy and precision are basic to nematode research and rational management decision. Taylor's Power Law is used to determine sample size. The published power law parameters are used here differently to define levels of reliability associated with a fixed, cost-determined, sample size. Such levels may provide the decision-maker with more informative data on sampling methods and their reliabilities. Instead of solving for unknown sample number, the rearranged equation to solve for sampling accuracy permitted the use of exact Student's t-values. The t-values for confidence limits and standard normal variate were used. Both statistical techniques resulted in identical confidence band, in terms of being below, at, or above the economic threshold level, in the majority of cases. Reckoning with new trends in collecting and processing samples, a 99% confidence interval above action threshold of *Tylenchulus semipenetrans* population with mean = 2500 at a precision of 80% or more would be attainable.

Keywords: Action thresholds, berseem clover, citrus, economics, nematode distribution, rice

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1 Introduction

Sampling of plant-parasitic nematodes for prediction purposes, their survey for ecological investigations and farming resource allocation, and small-plot sampling for experimental research have all been examined to optimize the costs of estimating nematode population levels. Therefore, different methods of nematode sampling and monitoring techniques and devices are adequately addressed (Been and Schomaker, 2013). Taylor's Power Law has been favored for its advantage and flexibility in the development of sampling plans, transformation of field data to meet assumptions necessary for parametric statistical analysis and satisfactory quantitative measurement of nematode spatial distribution. The power law by Taylor (1961) states that: the variance (S^2) of a population is proportional to a fractional power (b) of the arithmetic mean ($\bar{x}$): $S^2 = a\,\bar{x}^b$ or $\log S^2 = \log a + b \log \bar{x}$ (i), where a and b are population parameters; a depends chiefly upon the sample size and b is an index of nematode dispersion (Ferris, 1984; McSorley et al., 1985; Abd-Elgawad and Hasabo, 1995; Abd-Elgawad, 2014). This law is used to determine sample sizes to achieve a pre-determined level of sampling error for the nematodes (Duncan et al., 1989; Abd-Elgawad, 1991, 1992). In the present study, the nematode spatial parameters estimated in the latter three references are used differently. Rather than determining the sample size for a given level of precision of the estimate, I determined the reliability for a fixed, cost-determined, sample size for *Tylenchulus semipenetrans*, and *Hirschmanniella oryzae* in citrus and rice fields; respectively. Duncan and Phillips (2009) speculated that sampling programs reviewed by Barker and Imbriani (1984) remains largely current because general sampling and extraction methods have changed little in the subsequent years. More recently, however, a few developments have been made in these methods (Been and Schomaker, 2013; Van den Berg et al., 2014; Reid et al., 2015) which open new avenues for further options to optimize the above-mentioned costs. Therefore, this study uses previously published nematode distribution patterns to provide more informative

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Corresponding author: M. M. M. Abd-Elgawad, Ph.D., Phytopathology Department, Agricultural and Biological Research Division, National Research Centre, El-Tahrir St., Dokki 12622, Giza, Egypt. Email: mahfouzian2000@yahoo.com. Tel: 202-35460355, Fax: 202-33370931.

theoretical and practical data on different sampling and statistical methods and their corresponding reliabilities for different cropping systems. Also, more sampling precision levels are presented herein which may possibly be tailored with the recent advances and developments in processing soil sampling rate to a several folds increase. Their merit is statistically discussed herein especially when confidence interval of a population mean is not well above or below the action thresholds. Two nematode types with high and low economic threshold are addressed. In this context, the output for estimating sample accuracy and precision using the Student's *t* value versus standard normal deviate (*z*), previously reported by Duncan et al. (1994) was expanded herein for further comparison.

2 Materials and methods

The data base used in this study was obtained from published papers (Table 1) on the spatial distribution pattern of *Meloidogyne* spp. on berseem clover (Abd-Elgawad and Hasabo, 1995), *Tylenchulus semipenetrans* on citrus (Duncan et al., 1989; Abd-Elgawad, 1992), and *Hirschmanniella oryzae* in rice fields (Abd-Elgawad, 1991). Sample stratification in berseem clover and citrus in Egypt were based on previous crop yield and leveled divisions to control surface irrigation, respectively. Based on estimating coefficients for Taylor's Power Law (Taylor, 1961), these studies estimated optimum sample sizes using a pre-determined level of sampling error for the nematodes. Here, the reliability, i.e. the probability of achieving a specified degree of accuracy, of sampling plans for these cropping systems was evaluated. So, the unknown term (E = standard error to mean ratios, or D = confidence interval half-width to mean ratios) expressing reliability is solved. When parameters a and b of Taylor's Law are known, D can be found (Ferris, 1984) from: $n = (t_{\alpha[n\text{-}1]}/D)^2 a\,\bar{x}^{b\text{-}2}$ (ii), where n is the number of samples, $t_{\alpha[n\text{-}1]}$ is the appropriate Student's t value for confidence limits of $1–\alpha$ and $n–1$ degrees of freedom (Duncan and Phillips, 2009). Also, D or E can be estimated from the general formula: $D = t_{\alpha[n\text{-}1]}\,S/\bar{x}(n)^{0.5}$ or $E = S/\bar{x}(n)^{0.5}$ (McSorley and Dickson, 1991). The available fund to collect and process samples was divided by cost per sample unit to decide the number of samples that could be covered by a limited fund. This cost, numerically supposed in some cases, covers contributions of various activities to sampling nematode populations (Goodell and Ferris, 1981). Thus, number of samples and probability level ($1–\alpha$) are known but the reliability (E or D) is determined: $E = (a\,\bar{x}^{b\text{-}2}/n)^{0.5}$; $D = t_{\alpha[n\text{-}1]}\ E$ (Tables 2-5). Minimum required sample numbers were calculated at two levels of accuracy (E = 0.20 and 0.25) and regressed against their corresponding population densities of 1, 5, 20, 100, 300, 1000, and 3000 nematodes/sample for the afore-mentioned nematodes. Sample precision at 95%, 90%, and 80% probabilities was also estimated (McSorley and Dickson, 1991) for *Meloidogyne* spp. and *T. semipenetrans* population

Table 1 Sampling plans used in four different studies of phytonematode distribution patterns

Crop	Sampling plan	Ref.
Citrus	Samples were obtained from beneath 80 Valencia orange trees growing on sour orange rootstock in a bedded, flood-irrigated orchard in Florida, USA in temporal survey. Trees were monthly sampled in the first year of the survey and bimonthly sampled in the second year. Sixteen soil cores (2.3 cm d × 30 cm deep) systematically obtained from a circular, 40.7-m^2 area beneath each tree were composited into a single sample on each sample date. Each sample was mixed, and a 60-cm^3 subsample was processed on a Baermann funnel$^+$ for 48 hours to recover J2 and male nematodes in the soil.	Duncan *et al.*, 1989
Citrus	Two hundred and forty five citrus tree blocks in El-Tahrir Province, Egypt, each equal to 2.1 ha and bordered with raised soil burms, were sampled for soil stages of the phytonematodes. The blocks were 24-year-old but differed with respect to scion-rootstock combinations, edaphic conditions and management practices. Seven subsamples, each from a random tree, were taken with a hand trowel (ca 6 cm diam. × 30 cm deep) beneath the tree canopy at 1.5 m from the trunk and composited into a single sample representing a tree block (2.1 ha). In a second survey of *T. semipenetrans*, one soil and root core (ca 6 cm diam. × 30 cm deep) for each sample was collected from beneath the canopy of a single tree. Samples from seven trees were obtained from each of 24 adjacent 2.1 ha sites. An every K th (K = 3×5 = 15 citrus trees) systematic sampling was followed where the sampled tree located at the centre of the fifteen trees. In both surveys, a 250 g portion of each soil and root sample was analyzed. Soil nematodes were extracted with a modified sieving centrifugation technique*. Forty percent of each sample suspension was counted, corresponding to 100 g soil.	Abd-Elgawad, 1992
Rice	Nine Egyptian fields of irrigated rice were sampled for soil and root stages of *Hirschmanniella oryzae*. In each field, almost 4200 m^2 area was delimited to take six random samples. Each sample consisted of four soil subsamples collected from different locations within a field. Five fields were sampled for rhizospheric soil and four fields for roots. The fields differed with respect to plant age, edaphic conditions and nematicidal application. Eight samples were collected on seven occasions in the temporal survey. Soil samples were collected on four occasions; August 21, September 4 and 18 and at harvest. Root samples were taken weekly after the first three soil sampling times. Nematodes were extracted with the same technique* per a 250 g soil sample or with the funnel technique$^+$ from eight root systems as replicates.	Abd-Elgawad, 1991

Note: * Jenkins (1964); $^+$Christie & Perry (1951).

densities for the arbitrary case where this density is fairly above an assumed action threshold and still within the range of their published population means; i.e. $\bar{x}$ = 10 and 2500 for *Meloidogyne* spp. and *T. semipenetrans* as low and high action threshold, respectively. Based on published papers and experience, the presumed threshold is set at 4 second stage juveniles/100 gm soil for *Meloidogyne* spp. in berseem clover (Baltensperger et al., 1985; Abd-Elgawad, 1986) and at 2000 juveniles and males per 100 cm^3 soil in Florida (Duncan and Cohn, 1990) or per 100 gm soil in Egypt (Korayem and Hasabo, 2005).

Table 2 Percentage level of accuracy as defined in terms of the standard error to mean ratio (E) and the ratio of the half-width of the confidence interval^x to the mean (D) for random sampling of *Hirschmanniella oryzae* in irrigated rice fields of Egypt

Cost of Samples (US $) n	Finance-based umber of samples	Mean nematode count per sample^y	Level of accuracy/reliability^z	
			D	E
Taylor's power law parameters: a = 0.692, b = 1.99 in Egypt				
150	15	150	45%	21%
200	20	160	38%	18%
250	25	150	33%	16%
200	20	1	39%	19%
250	25	1	34%	17%
750	150	1	13%	7%

Note: ^{x} $t_{.05[15-1]}$ = 2.145; $t_{.05[20-1]}$ = 2.093; $t_{.05[25-1]}$ = 2.064; $t_{.05[150-1]}$ = 1.96 from common *t*-tables. ^{y} Based on a sample size of 250 gm soil (Abd-Elgawad, 1991). ^{z} The fractional values rounded up to nearest two decimals (i.e. percentage).

Table 3 Percentage level of accuracy as defined in terms of the standard error to mean ratio (E) and the ratio of the half-width of the confidence interval^x to the mean (D) for stratified random sampling of *Tylenchulus semipenetrans* in citrus orchards of Egypt

Cost of Samples (US $) n	Finance-based umber of samples	Mean nematode count per sample^y	Level of accuracy/reliability^z	
			D	E
Taylor's power law parameters: a = 0.83, b = 1.95 in Egypt				
100	10	800	55%	24%
150	15	100	45%	21%
150	15	1	50%	24%
200	20	1	43%	20%
250	25	1	38%	18%
750	150	1	15%	7%
200	20	2100	35%	17%
600	120	2100	14%	7%

Note: ^{x} t.05[15-1] = 2.145; t.05[20-1] = 2.093; t.05[25-1] = 2.064; t.05[120-1] = 1.98 t.05[150-1] = 1.96 from common *t*-tables. ^{y} Based on a sample size of 100 gm soil (Abd-Elgawad, 1992). ^{z} The fractional values rounded up to nearest two decimals (i.e. percentage).

Table 4 Percentage level of accuracy as defined in terms of the standard error to mean ratio (E) and the ratio of the half-width of the confidence interval^x to the mean (D) for systematic sampling of *Tylenchulus semipenetrans* in citrus orchards of Egypt

Cost of Samples (US $) n	Finance-based umber of samples	Mean nematode count per sample^y	Level of accuracy/reliability^z	
			D	E
Taylor's power law parameters: a = 1.175, b = 1.95				
100	10	800	66%	29%
150	15	100	54%	25%
200	20	1	51%	24%
250	25	1	45%	22%
750	150	1	17%	9%
200	20	2100	42%	20%
600	120	2100	16%	8%

Note: ^{x} $t_{.05[15-1]}$ = 2.145; $t_{.05[20-1]}$ = 2.093; $t_{.05[25-1]}$ = 2.064; $t_{.05[120-1]}$ = 1.98; $t_{.05[150-1]}$ = 1.96 from common *t*-tables. ^{y} Based on a sample size of 100 gm soil (Abd-Elgawad, 1992). ^{z} The fractional values rounded up to nearest two decimals (i.e. percentage).

Table 5 Percentage level of accuracy as defined in terms of the standard error to mean ratio (E) and the ratio of the half-width of the confidence interval^x to the mean (D) for random sampling of *Tylenchulus semipenetrans* in citrus orchards of Florida, USA

Cost of Samples (US $) n	Finance-based umber of samples	Mean nematode count per sample^y	Level of accuracy/reliability^z	
			D	E
Taylor's power law parameters: a = 9.2, b = 1.57				
200	10	800	52%	23%
300	15	100	62%	29%
400	20	1	142%	68%
500	25	1	125%	61%
3000	150	1	49%	25%
200	20	2100	27%	13%
600	120	2100	11%	5%

Note: ^{x} $t_{.05[15-1]}$ = 2.145; $t_{.05[20-1]}$ = 2.093; $t_{.05[25-1]}$ = 2.064; $t_{.05[120-1]}$ = 1.98; $t_{.05[150-1]}$ = 1.96 from common *t*-tables. ^{y} Based on a sample size of 100 cm^3 soil in Florida (Duncan *et al*., 1989). ^{z} The fractional values rounded up to nearest two decimals (i.e. percentage).

3 Results

Pre-defined sample costs provided a basis of estimating the sampling accuracy or reliability for *Hirschmanniella oryzae* in irrigated rice fields (Table 2), and *Tylenchulus semipenetrans* in citrus orchards of Egypt (Tables 3-4), and Florida, USA (Table 5). For example, the level of accuracy as defined in terms of the standard error to mean ratio (E) by collecting 20-150 samples in case of one nematode per sample ranged 7%-19% for *H. oryzae* (Table 2), and 7%-68% for *T. semipenetrans* (Table 3-5) according to the used sampling plan (Table 1). The levels of accuracy are set-up based on specific amounts of cash which are used as the costs of collecting, processing, and counting the nematodes. They are derived from agricultural extension in Egypt (M. M. Mohamed, The National Research Center, 2014, personal communication) and Florida (W. T. Crow, University of Florida, 2013, personal communication). These costs were generally based on sample plans; each consists of a collecting pattern, the number of samples comprising that pattern, and the number of composite cores/subsamples in the sample. In Florida (USA), invariable charge for analysis of soil/root sample is US $ 20. In Egypt, it was found that a charge frequently equivalent to US $ 10 is the cost of collecting, processing, and counting the nematodes in one sample for a minimum of 5 samples (5×10=US$ 50). This cost includes sample transportation to the identification laboratory. Yet, the prices depend on the number of samples; if the number of samples increases, the price per sample decreases to one-half for 50-150 samples and probably up to 70% discount for >150 samples. Such costs were adopted in the calculations for sampling costs in Egypt (Tables 2-4) and Florida (Table 5). Such costs progressively increased as sampling plan changed. For example, assuming level of one nematode (*T. semipenetrans*) per sample, 15, 20, and 150 samples should be taken in stratified random (Table 3), systematic (Table 4), and random (Table 5) sampling, respectively to achieve almost the same accuracy, i.e. E = 24%-25% according to the Power Law. Consequently, the costs are reduced from $ 750 (Table 5) to $ 200 (Table 4) and finally $ 150 (Table 3) using Egyptian prices. The former cost (Table 5) will rise to $ 3000 using USA prices.

A non-linear relationship was found as numbers of samples were regressed against seven different population densities of the above-mentioned nematodes at standard error to mean ratio (E) of 0.25 (Figure 1) and 0.20 (Figure 2). At any of these two specified reliabilities, the fewest number of samples is recorded when nematode population size is greatest for each of the plotted nematodes. On the other hand, at lower population level, the highest number of samples is recorded by *T. semipenetrans* of Florida probably because of the high intercept value of its power law (a = 9.2). For a given nematode density, the number of samples more markedly increased from E = 0.25 (Figure 1) to E = 0.20 (Figure 2), at the lower than higher densities. For example, at 20 nematode 100 cm^{-3} soil, the numbers of samples needed to achieve E = 0.25 and 0.20 equaled 41 and 63 samples whereas only 5 and 7 samples at 3000 nematodes 100 cm^{-3} soil, respectively, for *T. semipenetrans* of Florida orchards (Figures 1-2).

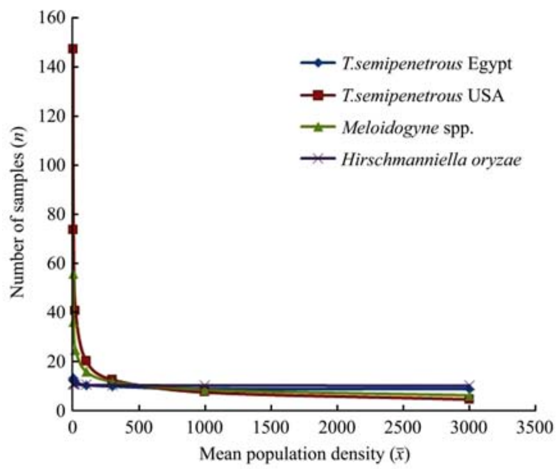

Figure 1 Numbers of samples needed to estimate the nematode population densities based on Taylor's Power Law parameters (a and b) at standard error to mean ratio (E) of 0.25. The parameter values from Abd-Elgawad (1992) for *T. semipenetrans* on citrus in Egypt, Duncan et al. (1989) for *T. semipenetrans* on citrus in USA, Abd-Elgawad and Hasabo (1995) for *Meloidogyne* spp. on berseem clover, and Abd-Elgawad (1991) for *H. oryzae* on rice are used in the equation $n=(E)^{-2}\, a\, \bar{x}^{(b-2)}$

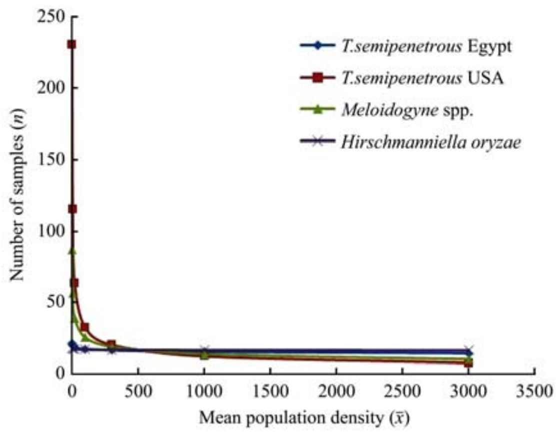

Figure 2 Numbers of samples (n) needed to estimate the nematode population densities based on Taylor's Power Law parameters (a and b) at standard error to mean ratio (E) of 0.20. The parameter values from Abd-Elgawad (1992) for *T. semipenetrans* on citrus in Egypt, Duncan et al. (1989) for *T. semipenetrans* on citrus in USA, Abd-Elgawad and Hasabo (1995) for *Meloidogyne* spp. on berseem clover, and Abd-Elgawad (1991) for *H. oryzae* on rice are used in the equation $n=(E)^{-2}\, a\, \bar{x}^{(b-2)}$

Precision for population density estimates of *Meloidogyne* spp. in berseem clover fields in Egypt and of *T. semipenetrans* in Egypt and Florida are shown (Table 6). For each plan, standard error to mean ratios and confidence intervals at three probability levels are calculated using the published power law parameters of these plans. The confidence intervals around the means of *T. semipenetrans* populations are generally broader for 99%, 95%, and 80% probability levels than for those of *Meloidogyne* spp. probably due to the high mean ($\bar{x}$ = 2500) of citrus nematode population used and the relatively low number of samples. Yet, Florida populations of the citrus nematode had narrower confidence band than those of stratified random which showed narrower confidence intervals than systematic sampling plan in Egypt. The appropriate Student's *t* value for confidence limits of 1 - α and n - 1 degrees of freedom was compared to the upper $\alpha/2$ point for the standard normal variate ($Z_{\alpha/2}$) from a table (2-tail) of probability α = 0.01, 0.05, or 0.20 (Table 6). Although Student's *t* value always had a higher figure than that of the standard normal variate especially at lower number of samples, both techniques were mostly comparable in maintaining the upper and lower limit of a confidence interval above the action threshold for *Meloidogyne* spp. populations. Their lower limits were equal to or slightly lower than the action threshold only at three cases of 99% confidence interval. If these cases are a cause of concern to achieve more accurate and precise management decision, they may be overcome by increasing sample number to 47 (Table 6). Only in one case at 80% confidence interval for *T. semipenetrans,* however, the use of both techniques permitted the management decision to be applied because this interval was above the action threshold. Probabilities of Student's *t* distribution (or the standard normal variate) and precision are functions of sample number, and therefore, it is clear that the breadth of any confidence band listed in table (6) becomes narrower for any of these plans as the associated probability decrease.

Table 6 Precision estimates for *Meloidogyne* spp. and *Tylenchulus semipenetrans* densities based on data derived from berseem clover fields and citrus orchards, respectively*

Nematode/Crop	Standard error to mean ratio (*E*)**	½ confidence interval to mean ratio (*D*)**	99% confidence interval^{+}	95% confidence interval^{++}	80% confidence interval^{+++}
Taylor's power law parameters: *a*		*n*	n strat e ran om sampl n ypt		
Meloidogyne spp./Berseem clover	0.25	0.51	3-17	5-15	7-13^{t}
	0.25	0.49	4-16	5-15	7-13^{z}
Taylor's power law parameters: *a*		*n*	n strat e ran om sampling, Egypt.		
Meloidogyne spp./Berseem clover	0.21	0.42	4-16	6-14	7-13^{t}
	0.21	0.40	5-15	6-14	7-13^{z}
Taylor's power law parameters: *a* = 3.483, *b* = 1.729; *n* = 47 10 in stratified random sampling, Egypt.					
Meloidogyne spp./Berseem clover	0.20	0.40	5-15	6-14	7-13^{t}
	0.20	0.39	5-15	6-14	7-13^{z}
Taylor's power law parameters: *a* = 0.83, *b* = 1.95; *n* in stratified random sampling, Egypt.					
*T. semipenetrans/*Citrus	0.17	0.35	1302-3698	1623-3377	1945-3055^{t}
	0.17	0.33	1421-3579	1679-3321	1963-3037^{z}
Taylor's power law parameters: *a* = 1.175, *b* = 1.95; *n* in systematic sampling, Egypt.					
*T. semipenetrans/*Citrus	20%	42%	1074-3926	1457-3543	1838-3162^{t}
	20%	39%	1216-3784	1523-3477	1861-3139^{z}
Taylor's power law parameters: *a* = 9.2, *b* = 1.57; *n* in random sampling, Florida, USA.					
*T. semipenetrans/*Citrus	13%	26%	1598-3402	1840-3160	2081-2919^{t}
	13%	25%	1688-3312	1882-3118	2095-2904^{z}

Note: * Abd-Elgawad & Hasabo (1995) for berseem clover, Abd-Elgawad (1992) for citrus in Egypt, and Duncan *et al*. (1989) for citrus in Florida. Soil sample size of 100 gm in Egypt or 100 cm^3 in Florida. ***E* and *D* values rounded up to nearest two decimals with *D* at 95% probability. ^{t} and ^{z} refer to using Student's t value and standard normal deviate, respectively, in a row, where $^{+}t_{.01[30-1]}$ = 2.756; $^{++}t_{.05[30-1]}$ =2.045; $^{+++}t_{.20[30-1]}$ = 1.311; $^{+}t_{.01[44-1]}$ = 2.695; $^{++}t_{.05[44-1]}$ = 2.017; $^{+++}t_{.20[44-1]}$ = 1.302; $^{+}t_{.01[47-1]}$ = 2.687; $^{++}t_{.05[47-1]}$ = 2.013; $^{+++}t_{.20[47-1]}$ = 1.300; $^{+}t_{.01[20-1]}$ = 2.861; $^{++}t_{.05[20-1]}$ = 2.093; $^{+++}t_{.20[20-1]}$ = 1.328; $Z\alpha/2$ = 2.576 for α= 0.01; $Z\alpha/2$ = 1.96 for α = 0.05; $Z\alpha/2$ = 1.282 for α = 0.20 from common statistical tables.

4 Discussion

This study addressed accuracy and precision as two substantial indicators that regularly used for judging the effectiveness and associated economics of phytonematode sampling. Their conscious use can greatly assist in realizing nematode population densities and applying relevant actions as necessary. Admittedly, the more the accuracy and/or precision level is, the higher the sampling cost becomes and vice versa.

Since the impact of sample optimization tactics on the detection of species occurring at low frequency in a field is of particular interest, reliability levels were reported herein mainly for nematode detection; one nematode per sample (Tables 2-5). Actually, detecting nematode infestations in samples of quarantine and certification programs usually employ the positive binomial distribution which is best introduced through the concept of probability. For the positive binomial, probabilities of pest detection require a pre-determined level of infestation or definite number of infested units in the examined plants, pots, soil, or cuttings that constitute the examined lot/nursery (Salama and Abd-Elgawad, 2003). On the contrary, knowledge of the dispersion parameter (*b*) of the power law for each of the nematode populations reported herein indicated their aggregated distribution (likely fit negative binomial) and allowed estimate of the soil sampling accuracy and precision. Interestingly, in large-scale surveys of 7-ha and 2.6-ha fields, a ten-core sample reliably detected most phytonematode species encountered where detection probability augmented with distance between cores because microhabitats which impact the nematode spatial dispersion were sampled more properly (Prot and Ferris, 1992). Sampling using a grid pattern with dimensions smaller than the focus in order to be optimized with nematode dispersion should more adequately detect phytonematodes and give proper weight to the larger, non-infested zone (Duncan and Phillips, 2009). On the other hand, nematode population density of 100 nematodes per sample presented herein as a numerical example does not exclude the fact that the action threshold density may be under (*Meloidogyne* spp.) or above (*T. semipenetrans*) this level. Therefore, minimum number of samples required for commonly encountered population levels are also considered at two reasonable reliability levels (E = 0.20; Figure 1 and E = 0.25; Figure 2).

Admittedly, caution should be used when using economic threshold figures because nematode population levels may affect crop yield differently under various conditions and locations. In a Florida orchard, the threshold was about 850 juveniles 100cm^{-3} during periods of low population growth but citrus yields were not measurably reduced if populations were below 2000 juveniles 100 cm^{-3} in the peak period (Duncan and Cohn, 1990), while the corresponding thresholds per 1 kg soil and 5 gm roots in Egypt were 13000 and 15000 nematodes during periods of low populations in February and August but 36000 nematodes during the peak period in March (Korayem and Hasabo, 2005). In Florida, late spring population levels of *T. semipenetrans* were recorded in the range of 2500-3000 nematodes/100 cm soil in a temporal survey (Duncan et al., 1989) which provide practical application of sampling precision associated with this survey in table (6). If so, sampling intensity should be increased to 40 and 53 samples to maintain confidence interval of 95% (2063-2937) and 99% (2001-2999) above the economic threshold in Florida using $Z_{\alpha/2}$. Similarly, to be above the threshold figure, 40 samples are needed for 80% confidence intervals of 2120-2880 and 2048-2952 in stratified random and systematic sampling plans of citrus in Egypt, respectively. These latter will be increased to 100 and 133 samples for 99% confidence intervals of 2018-2982 and 2002-2998, respectively using $Z_{\alpha/2}$. Given the great spatial heterogeneity observed in nematode population densities, McSorley and Dickson (1991) proposed 80% rather than 95% confidence interval to be attained more easily. In recent years, however, new sampling and extraction methods have been developed (Been and Schomaker, 2013), proficiency tests could provide added value through effective comparison of extraction processes used in various laboratories of European countries and useful employment of the accuracy and precision levels of the nematode counts could monitor and deliver trends and anomalies to the participating laboratories in order to achieve improvements (Van den Berg et al., 2014), and new high-throughput diagnosis of nematodes in soil samples has enabled several fold increase in the number of processed samples (Reid et al., 2015). Interestingly, European and Mediterranean Plant Protection Organization (EPPO) before 2010 detected the potato cyst nematode-standard focus with an average probability of 12% but new methods could detect this small infestation with 90% probability (Been and Schomaker, 2013). Moreover, in citrus orchards of Florida, the densities of organisms, including nematodes, at different trophic levels are measured by real-time qPCR. So, adopting a higher level such as 99% precision (Table 6) is timely especially because striking examples of faulty quarantine and certification programs have led to the spontaneous introduction and/or spread of nematode pests despite great efforts exerted on quarantine. Specifically, the importance of certification programs in citrus orchards has been well documented for both Florida and Egypt. Of special interest is the relatively high *T. semipenetrans* population level in the citrus nurseries of Nile delta, Egypt which would maximize the probability of their detection in these nurseries before transplanting the infested citrus seedlings to newly reclaimed areas with healthy soil.

On the other hand, several experiments revealed that the three most common species of root-knot nematode, *M. incognita*, *M. javanica* and *M. arenaria*, are primary limiting factors to berseem clover production which is severely damaged with as low as 4 juveniles/100 gm soil of newly reclaimed, non-fertile sandy areas of Egypt (Baltensperger et al., 1985; Abd-Elgawad, 1986). Berseem clover is the most important forage legume in Egypt due to its limited bloat troubles, ease of raising, relatively long growing season, and fast winter growth rate. Nevertheless, no chemical pesticides are usually applied during the lifetime of the berseem crop to avoid poisoning of cattle and livestock. Although growing satisfaction of sustainable agriculture involves enhancing the natural enemies of phytonematodes, it is difficult to rely on bio-nematicides as confidently as chemical nematicides. Bio-nematicides are frequently less effective and costly. Therefore, a stakeholder should better be sure that the confidence interval of the nematode population is above the action threshold figure prior to bio-nematicidal application in berseem clover fields. A 99% confidence interval with as relatively low precision as 40% of the true mean is valuable since most of nematode population rates were above management threshold levels for $\bar{x}$ = 10 (Table 6). This same level of precision was also considered worthy for sampling *T. semipenetrans* in a 2 ha areas of various Florida orchards since most of nematode population rates were well above or below management threshold levels (Duncan and Phillips, 2009). Moreover, the three sampling plans of *T. semipenetrans* with $\bar{x}$ = 2500 reported herein for citrus orchards with as high precision as 80% or more could maintain nematode population rates in the three tested probabilities of confidence intervals at or above management threshold level. Needless to remind that a high *T. semipenetrans* population density was assumed herein because confidence interval half-lengths as high as 100% or more in case of low population density will produce small population figures which can easily be separated from the high economic threshold. On the contrary, a low *Meloidogyne* spp. population level was proposed since its management threshold figure is low which generally requires greater accuracy (Duncan and Phillips, 2009).

On the other hand, the use of a Student's *t* value rather than a standard normal deviate (*z*) in formulae to estimate sample size could also increase the estimates by an average of three samples as reported by Duncan et al. (1994) who discussed cases in which the use of *z* rather than Student's *t* is convenient. Actually, they considered the Central Limit Theorem which implies that nematode counts from composite samples approach normality with increasing numbers of cores per sample. So, such an excess of 3 samples may be overlooked in relatively large samples especially when costs needed to estimate the true population, not sample, mean is considered. On the contrary, at small sample size, a three-unit increase may double or even triple the sampling cost if Student's *t* is used. Therefore, the aim of nematode sampling should be considered when calculating the required number of samples. Similarly, it is assumed herein that the larger the sample, the more the *t* distribution resembles a normal distribution. Thus, in case of small number of samples, $Z_{\alpha/2}$ values are used herein only for theoretical comparison with Student's t values (Table 6).

Several studies set a precision range of 75%-85% but there is no globally acceptable level. Thus, depending on the cost of the management alternative, the required number of nematode samples in tomato and cotton fields (Ferris et al., 1990) was several-fold higher than that of previous recommendations by Ferris et al. (1981) and would involve additional costs not factored into the calculations. Furthermore, precision level acceptable as a basis for nematode management decisions may vary greatly from one nematode species to another. Therefore, in case of rice (Table 2), and citrus (Tables 3-5) sampling where more than one pathogenic species was present in the rhizosphere, it would be more conservative to use a sampling plan for the nematode species requiring the highest accuracy in terms of number of samples. Other nematode species would then be sampled with even greater levels of precision. Goodell and Ferris (1981) speculated that precision levels of 50% are acceptable for *Merlinius*, but the variation of *Meloidogyne* estimates must be kept within 20% of the true mean. Hence, it may be more topical and informative to present different levels of accuracy and precision, as presented herein, to allow the decision-maker to select the best available option in certain cases than to estimate sample size optimization with a predetermined reliability.

Admittedly, an efficient sampling to estimate nematode populations in the field should be backed up by sound technical information (Ferris et al., 1981; Ferris, 1984; Duncan and Phillips, 2009; Been and Schomaker, 2013; Van den Berg et al., 2014) and consequent judgments for each specific case before being faced with a cost and sampling reliability tradeoff. Yet, survey of the population distribution of *T. semipenetrans* in citrus orchards showed a possibly unreal economic merit of stratified random over systematic which was in turn better than random sampling in terms of achieving comparable reliability (E=24%-25%) with less sampling costs (Tables 3-5). One might claim that because the tree blocks were divided into 2.1 ha areas in order to get leveled divisions to control surface irrigation in stratified random sampling, irrigation water in stratifications apparently played a role to fairly reduce variability among samples related to a stratified block which likely resulted in the lowest a value; the power law parameter for the sample size (Ferris, 1984; McSorley et al., 1985). In fact, Duncan et al. (1994) demonstrated a remarkable advantage of systematic over random sampling as well. Since roots of citrus trees and their associated *T. semipenetrans* numbers diminish with distance from the trunk, simulated sampling on a systematic approach lowered a considerable source of variability and decreased sampling size by about one-half. Differences in parameter a in stratified random, systematic, and random sampling are in the order: 0.83< 1.175<9.2, respectively (Tables 3-5) which documented the robustness of this parameter as index of sample size. Nevertheless, caution should be exercised because values of a vary greatly not only with sample numbers but also with location, plot size, and number of cores/subsample per sample (Abd-Elgawad, 1992). Hence, evaluation of sampling plans will vary greatly but should consider other factors such as root depth and distribution of the susceptible host, edaphic factors, degree of host susceptibility, sampling time/technique, extraction method/laboratory, and estimated/predicted crop loss as well as logistics and practicality of the required number of samples which may help in selecting a rational reliable level. The opposite opinion is that such a possibly false merit was quite apparent when used for nematodes at a detectable level, i.e. one nematode per sample. That is because base one raised to any power value equal one; that is, in our case $\bar{x}^{b-2} = 1^{-0.05} = 1^{-0.43}$, though the index of nematode dispersion $b = 1.95$ means that the population is more aggregated and approaches logarithmic distribution. Consequently, this population requires more samples than the second population with b = 1.57. Therefore, population with $b = 1.57$ had generally better precision than that with $b = 1.95$ at $\bar{x} = 2500$ (Table 6). Eventually, sample number is the product of both power law parameters and the precision level according to the above-mentioned equation (ii).

In conclusion, higher precision than commonly used one may be adopted especially when the management threshold figure of a nematode species lies within the confidence interval of the sample mean for the studied species. A number of developments in sample collection and process in recent years may facilitate such an adoption. On the contrary, if nematode population means, and consequently their confidence band, are well above or below economic threshold levels, there is no need to adopt a high precision level (Duncan and Phillips, 2009). Generally, best practicality of sampling cost and benefit tradeoff is gained when different levels of sampling reliabilities along with their corresponding costs are given (Tables 2-5). Such different levels of sampling *Meloidogyne* spp. in fields of berseem clover was recently investigated (Abd-Elgawad, 2016). A caution should be used, however, when using action threshold figures not only because nematode population levels may affect crop yield differently but also because different statistical techniques may sometimes produce inconsistent results (Tables 6).

5 Conclusions

Nematode sampling is an increasingly important component of plant disease diagnosis and control, especially with recent developments in molecular nematode identification and high-throughput diagnosis of nematodes in soil samples. Without adequate confirmation through sampling to cope with such developments, poor plant growth because of nematodes may be misinterpreted as nutrient deficiencies or other maladies. Adopting a higher level such as 99% precision or more is timely especially because striking examples of faulty quarantine and certification programs have led to the spontaneous introduction and/or spread of nematode pests despite great efforts exerted on quarantine. Also, the present study provided more informative theoretical and practical insights on different sampling methods and statistical techniques and their corresponding reliabilities for different cropping systems.

As a case in point, accuracy and precision of nematode sampling were investigated at different levels in developed (USA) and developing (Egypt) countries. The levels presented herein may be tailored with developments in processing soil sampling in a specific region as well as with the available fund. Moreover, their merit is statistically discussed especially when confidence interval of a population mean is not well above or below the action thresholds. Higher precision than commonly used one may be adopted especially when the management threshold figure of a nematode species lies within the confidence interval of the sample mean for the studied species. Eventually, an efficient sampling to estimate nematode populations in the field should be backed up by sound technical information and consequent judgments for each specific case before being faced with a cost and sampling reliability tradeoff.

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