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Sample Size for Testing 'White Tip Nematode' on Paddy Seeds

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ABSTRACT The distribution of white tip nematode *Aphelenchoides besseyi* on paddy seeds was studied to determine the optimum sample size for seed certification and for quarantine processing of paddy germplasm imported for research purpose. The nematode population on the seeds collected from naturally raised crop was found to be skewed, as the nematodes were concentrated on a 5% seeds mixed with a large number (≈ 95%) of nematode free seeds. Based on detection levels and statistical analysis for reliability of results, the optimum sample size for certification purpose was found to be 1000 seeds and for quarantine processing, observation of at least 50 seeds is necessary.

Keywords: White tip nematode, *Aphelenchoides besseyi*, sample size, seed borne diseases, detection level, plant quarantine, germplasm, seed certification.

Studies were carried out with an objective to determine optimum sample size to ascertain the absence/ presence of nematodes for issuing of phytosanitary/seed certificate. Globally white tip nematode of rice, *Aphelenchoides besseyi* Christie, 1942 is the most wide spread nematode; because of being truly seed borne. Its sparse distribution among the seeds of a lot allows its undetected movement along with the healthy seed. Such patchy aggregation of the nematodes on the seeds makes the job of seed certification agencies and quarantine nematologists difficult. The only known method to detect white tip nematode in seeds is by soaking them in water; where the seeds can not be reused for further research or sowing purpose. The examination of still smaller sub-sample drawn from already small and valuable seed lots of germplasm collections does not always reveal the precise and true picture of seed borne nematodes.

Among annual crops the global spread and total damage caused by the seed borne nematode, *Aphelenchoides besseyi* is only next to that caused by stem and bulb nematode (*Ditylenchus dipsaci*). The nematode is known to have a wide host range and several races and has been reported from almost all paddy growing areas of the world (including Africa, Asia, Eastern Europe, North, Central and South America and the Pacific Region).

Plant Quarantine scientists examine samples for pests or pathogens with the responsibility to restrict entry of any alien species. The amount of seeds available for testing in plant quarantine laboratories is often less than the quantity generally available in seed testing labs. Thorough mixing of seeds and sub-sampling is not a problem while handling germplasm; it is the sample size which matters in case of tests where seeds are valuable as well as a few in numbers and especially when not reusable after the laboratory testing is over. So study was carried out to understand the actual nematode distribution among the seeds in a sample which affects the efficiency of their detection in any consignment. As a routine test for detection of plant parasitic nematodes a few seeds out of the original sample are soaked for 48 hours and suspension is examined under binocular. To meet the objectives, the detection technique should be efficient to the extent possible so that no seed borne pathogen is able to cross the international boundaries using seeds as via media. Therefore it is essential that the sample size is chosen correctly as this determines how well the test results relate back to the whole seed lot.

MATERIAL AND METHODS

A paddy seed lot, collected from Cuttack, India; infested with *A. besseyi* was soaked (randomly selected seeds) at 25°C and examined by submerging one seed individually or in batches (10, 50, 100, 1000, 5000 and 10,000 seeds) in ten replicates, in 50 ml to 1,000 ml beakers containing sufficient water for

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Table 1. The nematode emergence after 48 hours of soaking of seeds in water at 25°C

No. of seeds	Average number of nematodes emerged (calculated for 100 seeds with 10 replications)	Coefficient of deviation (CD)	Coefficient of Variance (CV %)
1	0	0	0
10	11.00	27	245.45
50	31.80	28.52	89.71
100	26.80	25.09	93.63
500	30.00	4.09	13.66
1000	27.08	0.59	2.20
5000	26.40	0.26	1.00
10000	26.25	0.17	0.67

Table 2. Nematode emergence between 48 to 96 hours of soaking of seeds in water at 25°C

No. of seeds	Average number of nematodes emerged (calculated for 100 seeds with 10 replications)	Coefficient of deviation (CD)	Coefficient of Variance (CV %)
1	0	0	0
10	9.00	22.3	248.17
50	11.00	22	200.13
100	14.50	17.6	121.52
500	27.26	4.7	17.10
1000	26.17	1.1	4.20
5000	26.43	0.3	1.12
10000	26.26	0.2	0.68

periods ranging between 48 and 96 hours. At each time interval, the contents were agitated and water poured into a separate clean beaker. The seeds were rinsed again with water, which was then added to the suspension. The suspension was passed through 400 mesh sieve to reduce the volume to 40-50 ml. The nematodes that emerged out in water suspension in 48 h were separated and seeds re-soaked in fresh water for another 48 hours. The extract was poured into a counting dish and examined under a stereoscopic microscope. All extracts were examined on the same day as it becomes cloudy with storage. For studying number and calculating percentage of seeds infected in the experimental lot of seeds experiment was repeated with 300 replicates as 10 replicates were not sufficient for reliable results. So, one seed per glass vial was soaked in 300 vials of 10 ml capacity. The suspension was observed for nematodes after 48 hours. Results were analyzed statistically to speculate number of infested and nematode free seeds; and the distribution of nematodes among the seeds in the lot to determine optimum sample size.

RESULTS AND DISCUSSION

The nematodes which emerged out from the seeds after soaking for 48 h or 96 h were counted and data showed that there is uneven distribution of nematodes in seeds (Table 1 and 2). Out of 300 glass vials each containing single seed in water, the nematodes emerged out from seeds in 14 vials, while rest of the seeds were free from the nematode contamination. Thus approximately 5% of the seeds were infested in the tested lot and rest of the seed were nematode free. Analysis reveals that when the sample size is 100 seeds the chances of detection of the presence of the nematode in the seed is just 10%.

When the sample size is 1,000 seeds or more the results are 95% reliable. The detection level is 60 % when the sample size is 500 seeds. Hence, the nematodes are not randomly distributed. At each interval, most of the *A. besseyi* recovered were fourth stage juveniles and due to lack of oxygen they tended to be motionless.

The number of nematodes emerging out in first 48 hours was not significantly different from the ones emerging out in next 48 hours. The nematodes continued to emerge even after 120 hours though counting of nematodes was not carried out beyond 96 hours due to fermentation of starch released from the seeds, in the water suspension.

Up to 14 nematodes were found on a single seed where they remained coiled up inside the palea. On paddy, nematode infested seeds are the primary source of contamination/ inoculum. When the crop is sown the nematodes become active and move to the growing points of leaves and stems where they feed. The nematode is known to survive in a state of anhydrobiosis for several years on stored paddy seeds but much less under field conditions. Thus the pest has adapted itself to be carried away with seeds. EPPO has listed *A. besseyi* as an A2 quarantine pest [1]. EPPO further recommends that rice seed from infested countries should be thoroughly tested.

In onion seeds *Ditylenchus dipsaci* was found to be not distributed randomly [2, 3]. When mixture of such seeds is sown in the fields, it results in the patchy distribution of the inoculums in the field and consequent patchy establishment of infestation in the soil. And ultimately the harvest again represents the skewed distribution of healthy seeds mixed with a few nematode infested seeds.

Even when initial inoculum levels are low, diseases such as bacterial blight in pea, anthracnose in lupins and 'aschochyta blight' in chickpea can hamper production under the favorable seasonal conditions. Pereira and Santos [4] observed that in one out of two samples of *Vicia faba* (of 50 seeds) 20,035 larvae were present while the other sample from the same lot was not having any *D. dipsaci*. Steiner and Lamprecht [5] found similar results when they observed that among the 9 % of the positive samples, only 3% of seeds were infected.

It is difficult to predict growing season conditions; however seed tests (along with suitable treatment) provide a useful via media against disease outbreaks even under favorable situations. Seed testing establishes the true disease risk associated with seed lot. Therefore the number of seeds tested and method used are the basis of predicting the crop losses at the minimum cost.

The analysis depicts that the great difference between the mean and variance of numbers of the nematode emerging out of sample size 1 to 100 seeds and the experiment with one seed each in 300 vials, make us conclude that the distribution of nematodes among seeds is not random. The distribution shows further that there are large number of seeds with no nematodes, mixed with few having infection, so distribution is skewed. Such a biological model is similar to Poisson log normal distribution [6]. Optimum sample size for providing true interference of the quantitative assessment of nematodes is 1,000 seeds. Same number of seeds should be soaked for certification and also where sufficient number of seeds is available. Skewed distribution of the nematodes among the seed lots poses difficulty in nematode detection in laboratories. Analysis of results for detection level, it is suggested that Quarantine Laboratories should give prophylactic treatment to all paddy germplasm wherever numbers of seeds soaked and examined per sample are less than 50.

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REFERENCES

1. EPPO, (1981). Data sheets on quarantine organisms' No. 122, *Aphelenchoides besseyi*. *EPPO Bulletin* **11**(1).
2. GOODEY, T. (1943). The aggregation of species within spatial units. In: *Statistical Ecology* Ed. G.P.Pa; F.C. Pielou and W.E. Waters. Pennsylvania State University Press **1**: 311-334.
3. SEINHORST, J.W. & J.L. KOERT (1969). Stengelaaltjes op uienzaad. *Gewasbescherming* **2**: 25-31.
4. PEREIRA, MC.L.S. & M.S.N. de A. SANTOS (1975). Occurrence of stem nematode (*Ditylenchiis dipsaci*) in seeds of bean (*Vicia faba*) in Portugal. *Ciencia Biologica Portugal* **2**(3): 85-88.
5. STEINER, A.M. & H. LAMPRECHT (1983). Infection of certified seed of *Vicia faba* with fungal diseasae *Ascochyta fabae* and the nematode *Ditylenchus dipsaci*. *Landwirtschaftliche Forschung* **36**(3-4): 198-206.
6. CASSIE, R.M. (1962). Frequency distribution models in the ecology of plankton and other organisms. *Journal of Animal Ecology* **31**: 65-92.