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Population dynamics of foliar nematode (*Aphelenchoides besseyi* Christie) infesting Tuberose in West Bengal

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Abstract
An experiment was conducted at BCKV Research Farm, Gayeshpur, during 2013-2016 to perceive the population fluctuation with references to the weather changes and to observe the changes of population in percentage of foliar nematode, *Aphelenchoides besseyi* Christie in tuberose. It was found that population dynamics played an important role during the changes occurred in the plants. Monitoring on nematode population during the plant growth period (*cv.* Calcutta Double) revealed that *A. besseyi* maintained maximum population during July month of rainy season that coincided with the start of heavy flush of tuberose. The least population was observed during December to February when average temperature, total rainfall and relative humidity remained quite low. Amongst the weather factors the nematode population had a positive correlation with Maximum temperature, minimum temperature, Rainfall and relative humidity in infected flowers.

Keywords: Foliar nematode, tuberose, population dynamics

1. Introduction
The cultivation of *Polianthes tuberosa* has become complex due to foliar nematode *Aphelenchoides besseyi* Christie infection that damages a high proportion of the plants. The foliar nematode *Aphelenchoides besseyi* Christie has been found to cause serious damage to this crop in WB and Orissa, consequently the earning of the flower growers have been reportedly reduced by about 50% (Mukhopadhyay 1997) [1]. The foliar nematode (*A. besseyi*) is one of serious concerns. The infection of nematode species induces a floral malady symptom in tuberose (Chakraborti and Ghosh 1993) [2]. This nematode species can cause yield loss up to 59% (Pathak, Khan 2009) [3] and has been considered as a key pest of tuberose (Khan 2006) [4], (Khan, Pal 2000) [5], (Khan *et al.*, 2012) [6]. This nematode mainly affects the floral part and the scape part of the tuberose plant. The nematodes first attack the leaf but very less and then move to the scape and at last aggregated in the floral parts where they feed vigorously. Infestation of foliar nematode in West Bengal is a potential threat for its profitable cultivation; the area under tuberose cultivation started declining on account of severe infestation of foliar nematode on the traditional cultivars like Calcutta Double. This nematode can easily disperse by means of infested bulb, irrigation water and farm implements (Khan *et al.*, 2012) [6]. Holtzmann (1968) [7] first reported this nematode in leaves of tuberose from the Hawaii Islands and further reported from Ranaghat region in West Bengal (Khan 2001) [8] and in Odisha (Das *et al.*, 2011) [9]. The nematodes population was influenced by the high humidity and high temperature, therefore the research was performed in BCKV Research Farm, Gayeshpur, during 2013-2016 to perceive the population fluctuation with references to the weather changes and to observe the changes of population in percentage.

2. Materials and Methods
The field experiment was carried out from 2013 - 2016 on *cv.* Culcatta double at Central research farm of Bidhan Chandra Krishi Viswavidyalaya, Gayeshpur, Nadia, West Bengal, located at 230N latitude and 890E at the elevation of 9.75 meters from mean sea level. The tuberose cultivar Calcutta Double was sown following all the recommended cultivation practices and the field and was naturally infested by the foliar nematode.

2.1 Collection of the flower sample
Nematode infested flower samples of 20g were collected randomly from fixed plots at one 15 days interval throughout the growing seasons and brought to the laboratory for extraction of nematodes. In laboratory, the collected samples were chopped into small pieces with the help of a sharp knife. Then, the chopped flowers were placed on wire gauge fitted over a petri plate containing clean water just touching the bottom of the wire gauge assembly. The assembly were covered with another petridish to prevent the water loss and was kept undisturbed for 12 hours. During the period, the nematode came out from the chopped flower materials and migrated freely in the water. Then, the prepared suspension was passed through 20 and 400 mesh sieves serially under tap water for cleaning the remaining plant toxic substances released and nematodes were collected in a beaker from the residues.

2.2 Killing (Water Bath Method) and fixing
The nematode suspension collected in the above mentioned process was taken in a beaker and water was heated in a separate container to a temperature of 600C. The beaker containing nematode suspension, was plunged in hot water at 600C and shaken continuously for distributing the nematode as well as uniform heating of suspension for 2-3 minutes.

Subsequently, the killed nematode were fixed by adding equal volume of 4% formaldehyde and were kept separately in the labeled plastic bottle for further study.

2.3 Estimation of nematode in samples and counting of population of the nematode
Plastic bottle containing fixed nematode suspension was stirred well by blowing through a pipette to ensure uniform distribution of the nematodes in the suspension before counting. Then 2 ml of the thoroughly stirred suspension was drawn and taken on counting disc thrice for counting the nematodes under stereoscopic binocular microscope. Thus, average number of foliar nematode in one ml of suspension was determined. To estimate the total population in 20 g of flower samples, the foliar nematode population in one ml suspension was multiplied by a factor of total volume of the suspension.

2.4 Climatic condition during the period of experiment
The data of average fortnightly temperature, relative humidity and rainfall during the entire period of experiment were collected from the record of the local Meteorological observatory of the All Indian Co-ordinated Research project on Agricultural Meteorology, Research Complex Building, Bidhan Chandra Krishi Viswavidyalaya, Kalyani, Nadia.

2.5 Statistical Analysis
Data collected during the period of investigation was subjected to statistical analysis Regression and Correlation of nematode populations with the weather data. The regression equation was generated using SPSS software.

3. Results and Discussions
Foliar nematode (A. besseyi) is mainly affected by the variation of weather factors viz., temperature, relative humidity (RH) and total rainfall. During the first year of the infected crop, maximum population was recorded during August 2013 (27032.69 nematodes per 20g flower sample) and the least population was recorded during November 2013 (22096.99 nematodes per 20g flower sample) (Fig 1). The population was found to be less at the first year of the crop during November 2013.

During 2014, the lowest and highest populations were recorded respectively in January and August, 2014. The population got smaller during the winter periods in December- March with the shrinkage of the plant vigour and less humidity and low temperature. The population then started increasing with the increase in temperature and humidity and attained the highest peak during July and during August where the temperatures and humidity were high. It attained the peak or highest population during August 2014 9 (2609.57 nematodes per 20g of flowers) during 2014 (Fig 1).

The population was significantly increased during the periods of 2015 with the increased in weather parameters. We could find that the population was increasing when the temperature and humidity rose. During the 2nd year we could find that the population of nematodes increased in 2nd year crops as compare to 1st year crops.

In 2016, (Fig 1) the highest population was found in the month of July (113713.63 nematodes per 20g of flowers) and the least population was found in the month of Jan (10003.02 nematodes per 20g of flowers) in infected flowers. Here, the differences in population between the infected flowers during the different months could be observed with huge quantity. Therefore, we could find that the nematodes populations were found to be higher in mostly summer seasons with high temperature, high humidity and rainfall.

This study demonstrated the fact that foliar nematode population exhibited positive correlation with the maximum temperature, RH and rainfall (Table 1). This was further evident from the observation during period of winter months under West Bengal conditions; the least population of *A. besseyi* was observed during winter months when temperature and RH in atmosphere were relatively low (Table 1). The decline of nematode population during September–November was primarily due to senescence of tuberose plants. Khan and Ghosh (2011) [10] also reported maximum foliar nematode population on Calcutta Single during July to November, while Das and Swain (2013) [11] recorded maximum population on Calcutta Single during the month of July to September. Bala (2007) [12] also concluded that the *Aphelenchoides besseyi* reached the peak during July in Calcutta double. Our finding also corroborates the observation of Khan (2004) [13] Therefore from this we could conclude that the nematodes' population growth was mostly dependent on weather parameters.

4. Conclusion
Intense observation on the population dynamics of the foliar nematodes in tuberose plants revealed that the population dynamics played an important role during the changes occurred in the plants. Intensity of infestation and percent infested plants were found greater during the periods of higher nematode recovery from flowers (27329.75 to 134018.78 nematodes/20 g of flower) which synchronised with the monsoon months. Activity of nematodes increased during the months of April - October. We could find that the population of nematodes increased with the increase in weather parameter (temperature, relative humidity and rainfall). It was also found that the nematode population had a positive correlation to the mentioned ecological parameters (Maximum temperature, minimum temperature, Rainfall and relative humidity) in nematodes infected flowers.

Table 1: Correlation and regression Coefficient of foliar nematode, *Aphelenchoides besseyi* in tuberose with weather factors during 2013-2016.

Weather parameters	Correlation(r)	Regression Equation
Max. Temperature (0C)	0.57	y = -631.28x2 + 44480x - 718939 R2=0.3718
Min. Temperature (0C)	0.72	y = 188.95x2 - 2600.7x + 15237 R2=0.4573
Relative Humidity (%)	0.50	y = 32.431x2 - 2556.8x + 44935 R2=0.2704
Total Rainfall (mm)	0.60	y = -0.2156x2 + 230.28x + 26653 R2= 0.3793

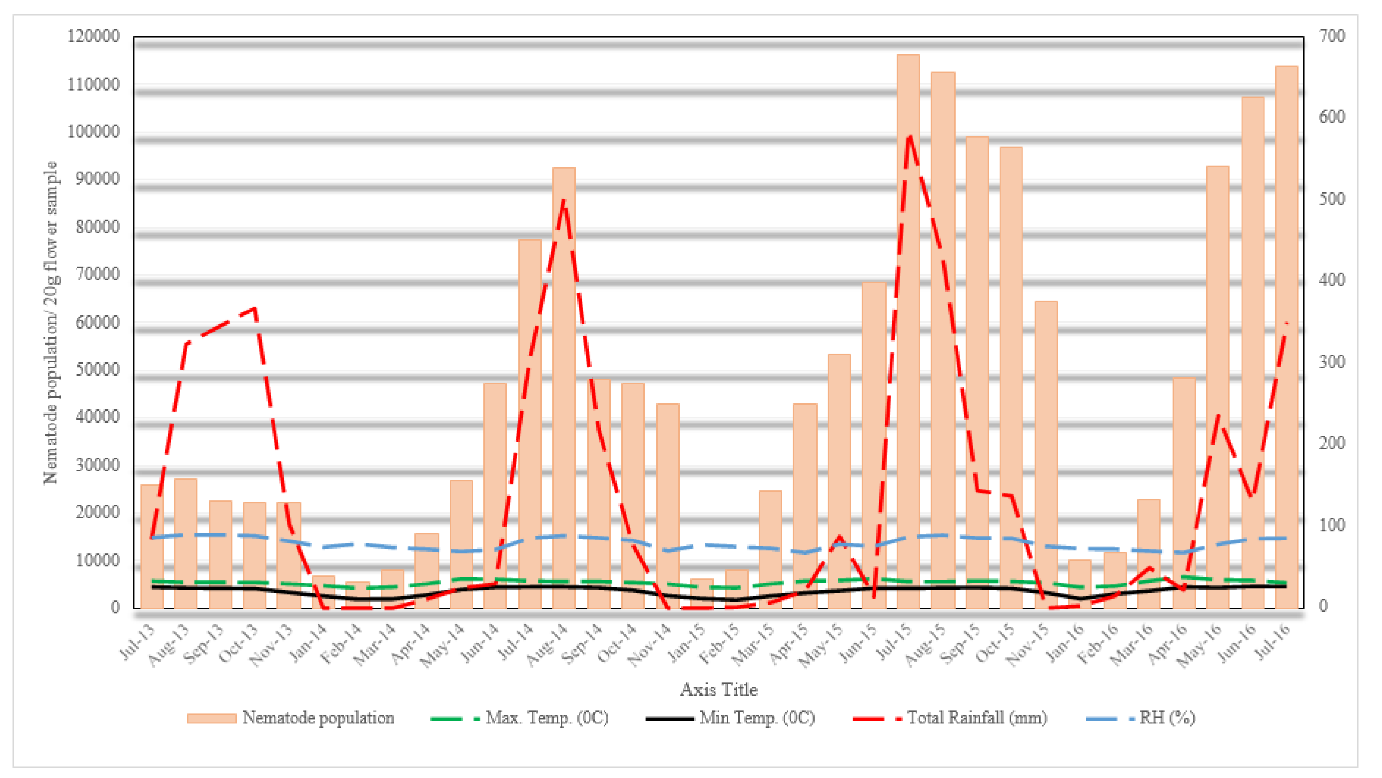

Fig 1: Population fluctuation of *Aphelenchoides besseyi* infecting tuberose (*cv.* Calcutta Double)

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