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Study on the symptomatology of floral malady of tuberose associated with foliar nematode infestation on the plants

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Abstract
A field experiment was conducted at central research farm, Gayespur, BCKV in the year 2013-2016 to observed the various symptoms produce in tuberose due to feeding by major pest i.e *Aphelenchoides besseyi* (foliar nematode) of Tuberose. Progressive manifestation of symptoms in infested tuberose stalks and flowers was closely observed continuously right from emergence of the stalk head to harvesting of flower. Symptoms development could be seen right from the early stages of the tuberose plants which continued till harvest of the plants. Close vigilance on the external symptoms revealed that all the yield attributes, *viz*., the length of stalk, spike, individual flower, weight of individual flower, weight of spike and numbers of florets were very less in infected tuberose plants (cv: Calcutta Double) than that of healthy looking ones. Symptoms usually first appeared on the leaves with initially marked by emergence of yellowish green or pale green or pale green leaves. The first visible morphological change on the flower stalk (scape) was in the form of prickle like structures of variable numbers on the surface of the floral stalk along the length which developed from the epidermis and with aging pricked on touch. Sometimes spike developed but the flowers did not bloom whereas, development of apparently normal spike with partial blooming of either lower most or the top most few flowers was also observed.

Keywords: Tuberose, foliar nematode, *Aphelenchoides besseyi*, symptomatology, West Bengal

Introduction
In every phase of life's celebrations, festivals and prayers, flowers hold a special position. Among these flowers, one of the most used is tuberose which is popularly known as "Rajanigandha", Tuberose (*Polianthes tuberose* L.), is the bulbous ornamental crop and has gained considerable importance in the world market. It is commercially cultivated for its various uses like decoration of floral ornaments, perfumes, beautification of home gardens and as cut flower in many countries of the world like Vietnam, China, Brazil, Italy, Iran, UK, USA etc. including India. The flower oil contains methyl benzoate, methyl anthranilate, benzyl alcohol, butyric acid, eugenol, nerol, farnesol, geraniol. About 2,110 ha are cultivated in West Bengal and rank first both in areas and production [1]. In West Bengal, the cultivation of tuberose is mainly confined in Panskura, Kolaghat and Debra of Midnapore (East and West), Ranaghat and Haringhata area of Nadia, Rajarhat of 24-Parganas (North) and Bhangar-I of South 24-Parganas districts. The plant parasitic nematode (PPN) is one of the most important pests attacking tuberose and is causing barrier to quality and quantity of flower production. Among the PPN, the most important were foliar nematode and Root Knot nematode infecting the tuberose crop. 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%. The nematode *Aphelenchoides besseyi* cause serious problem in tuberose by reducing almost 50% of earnings of the flower growers of the state [2]. The nematode was reported first time from the leaves of tuberose from Hawaii Island [3] and on rice from Madhya Pradesh (India) [4]. Subsequently, it was recorded from Ranaghat region of Nadia district [5] to cause severe loss due to malformed flowers. Tuberose bulbs were the primary source of inoculums where the nematodes continued to maintain their population till harvest of the crop [6].

Materials and methods
A regular study on the tuberose plants right from the emergence of stalk head to harvesting of flower was done.

30 plants were planted in each plot (size: 3 × 1.5m^2) with the spacing of 50 × 37.5 cm. Plants with the emerged stalk head were selected randomly and tagging of plants were done using aluminium tag and tagged plants during the programme were examined thoroughly to see any changes in colour, texture, characteristics of the leaves, floral scape and flower. The visual symptoms and gradual changes in the tuberose plants were recorded.

Results and discussions

Diagnosis of symptoms is considered the most important part in adopting any strategy for managing the nematode pests. Progressive manifestation of symptoms in infested tuberose stalks and flowers was closely observed continuously right from emergence of the stalk head to harvesting of flower. The plants were tagged by the aluminium tags at the time of emergence of the stalks and the progress of symptoms was observed keenly in every week with the growth attributes of the plants. The plants at emergence could not be differentiated as infected by the nematodes or healthy and as such all were tagged randomly. The observation was also taken according to the growth of the plants as the symptoms development was found to be different in different ages of the plants. Symptoms development in tuberose plants due to feeding by the foliar nematodes could be seen right from the early stages of the tuberose plants which continued till harvest of the plants. A comparative account of the unaffected ones was also maintained. Usually from the late first fortnight of stalk head emergence, the symptoms of nematode infestation on stalk and flower buds could be ascertained (Plates F and G). The leaves as well as the flower heads emerged out of the top portion of the bulbs where from maximum number of nematodes in bulbs was recovered. The tiny organisms while feeding entered in developed flower head and also fed on the epidermal layer of the newly formed stalk. Close vigilance on the external symptoms during the programme 2013-2016 revealed that all the yield attributes, *viz*., the length of stalk, spike, individual flower, weight of individual flower, weight of spike and numbers of florets were very less in infected tuberose plants (cv: Calcutta Double) than that of healthy looking ones.

Symptoms usually first appeared on the leaves. It was initially marked by emergence of yellowish green or pale green or pale green leaves. This might be due to reduction in chlorophyll content of the leaves following nematode infestation. Upon aging, the leaves started turning brown due to the secondary infection by other pathogens [7] where in conjunction with fungus nematodes became a very serious peril to the plants. In leaves, the discoloration could be seen at the base part and spreading up to the tip of the leaves. At last, the tips of most of the leaves assumed brown whiptail appearance. The browning was found in the midrib of the leaves too. These brown leaves most cases turned into brick colour and necrosis was noticed. Initially light chlorotic lesions developed along the mid axis (rib) of the leaf starting from the base and extended upwards, but never touched the apex subsequently became yellowish and finally turned brown or black and elongated necrotic spots [8]. All these mentioned observations corroborated our ones.

The first visible morphological change on the flower stalk (scape) was in the form of prickle like structures of variable numbers on the surface of the floral stalk along the length which developed from the epidermis as a result of nematode infection and with aging pricked on touch (Plate D). This was substantiated by the fact that the flowers of inflorescence having such morphological abnormalities also exhibited change in floral morphology and presence of nematode. The stalk was also easily brittle due to this nematode infestation. Proportionate to the degree of infestation at the initial stage, the distinctive symptoms could be ruggedness and twisting in the stalk in addition to the presence of mentioned prickles which all usually started appearing from late 2nd week after emergence of stalk head (Table 1) and the bracts were often found crinkled. The plants thus infested at early stage, suffered seriously from retardation of growth and remained stunted ultimately, the stalk length being 46.11-77.94 cm comparing to 82.50 cm- 105.42 cm of the healthy plants during the periods of maximum activities of the nematode observed in 2013-2016. Sometimes the stalk appeared to be distorted in shape and not straight. Brownish patches developed on flower stalk and also on flowers, tepals of the flower became thicker, anther became blackish brown in colour. The pedicels were also shortened in length in infected flowers as compare to healthy flowers [Plates: A and B]. The flowers of such plants remained very small, unopened, crinkled, very few in numbers. The stalk length, spike length, numbers of flower, spike weight, length and weight of individual flower were almost double in healthy tuberose crops comparing to those in infected tuberose crops of same age. The values of these yield attributes during the periods of observations were 46.11-77.94 cm, 12.49-21.97 cm, 13.39-24.35 numbers, 7.68 g – 24.30 g, 1.09 -3.04 cm and 0.45 g - 1.13 g respectively for stalk length, spike length, numbers of flower, spike weight, length and weight of individual flower (Table 2 and 3). On the contrary those values in unaffected plants were 82.50 cm- 105.42 cm, 28.50 cm -43.87 cm, 28.11 - 48.75 numbers, 66.25 g – 154.54 g, 3.65 cm – 6.21 cm and 2.06 g – 4.10 g respectively for stalk length, spike length, numbers of flower, spike weight, length and weight of individual flower (Table 2 and 3).

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 in the month April, May, June, July, August, September and October.

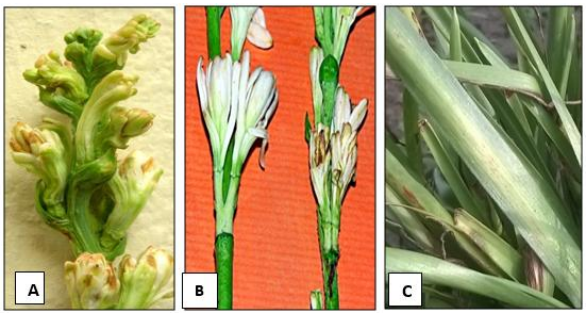
Degree of infestation by the nematodes in terms of development of spike and blooming of flowers were manifested differently. Sometimes spike developed but the flowers did not bloom whereas, development of apparently normal spike with partial blooming of either lower most or the top most few flowers was also observed (Plate E). In few cases the flower stalks emerged with a blind flower head where no spike developed. The infected flowers were hard, small, less in number and twisted. The browning discolorations were also found in the flower. The flowers also appeared to be in unthrifty way. In the severe condition, the flowers too were found to have prickle like structures in the outer petal. Healthy *Polianthes tuberose* plants produce stalk approximately 1 m in length and decorated with up to 40 white, blemish-free florets. In contrast, diseased plants produced stunted stalks bearing florets those were yellowed and browned. Market value of the flowers greatly reduced due to wrinkling of flowers and reduction of fragrance.

In some cases morphological abnormalities on the surface of scape could be observed without visible symptoms of abnormalities in the floral parts indicating presence of nematode. This observation suggests that there was a close

relationship between presence of the nematode and simultaneous development of some morphologically visible structures almost looking like spiny structure. This conclusion is drawn on the basis of the observation that the tuberose inflorescence having no visible symptoms of infestation due to attack of nematode did not bear any epidermal outgrowth on the scape. The nematodes population could be found in apparently unaffected plants also but due to its meagre in numbers, the symptoms appearance was not visible. The nematodes invaded the flower bud ectoparasitically, pierce the stamen, stigma as well as ovary before anthesis and resulted crinkled flower [5]. The infected flower stalk appeared rough, crinkled, stunted and finally distorted [9]. Brown streaks appeared on leaf bracts and petals and subsequently developed rusty brown spots. The severely infected flower stalk became rotten and brittle over drying. Their number per stalk was also reduced. Flowers became small, crinkled and distorted which were not acceptable in the market. All these findings were in conformity with the observations of the present author.

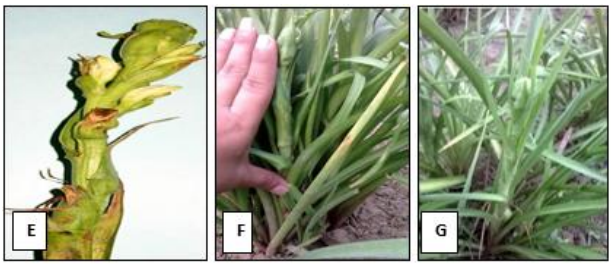
Conclusion

From the present study it can be concluded that the symptoms caused by *Aphelenchoides besseyi* started from the leaves to scape (flower stalk) and ended in the flower spike. Tuberose bulbs were the primary source of inoculums where the nematodes continued to maintain their population till harvest of the crop. The nematodes congregated at the top of the infected bulbs and entered in the developing flower head. Extensive feeding by the nematodes on the epidermis of the flower stalk made the surface rugged (prickle) along the length. The mostly infected parts in tuberose plants were flowers as most of the nematodes' aggregation was found there. Plants severely infested by nematode were mostly recognized by severely stunted growth and presence of profuse prickle like structures on the scape and flower, undulated scape surface and hardy brown flowers. To have a formulation of an economic and executable foliar nematode management to prevent the drastic economic lose of tuberose crop; we need the thorough understanding of the symptomatology of the crops.



Plates 1: A: Stunted, Crinkled, and hardy flower with shortened pedicel and twisted bract, B: Comparison between healthy pedicel and infected pedicel of tuberose flower stalk, C: Discoloured Infected tuberose leaves

Plates 2: D: Spiny outgrowth (prickle) or rugged surface on the scape of the nematode heavily infested tuberose crops (cv. Calcutta double)



Plates 3: E: Partially bloomed and unopened flower of Tuberose, F and G: Prickle on the scape during early age of the tuberose showing heavy infestation (cv. Calcutta double)

Table 1: Changes of growth attributes of infected tuberose plants and healthy tuberose plants during 2013-2016

	Infected Plants						Unaffected Plants					
Age of the crops	**Stalk length (cm)**	**Spike length (cm)**	**Length of individual flower (cm)**	**Weight of individual flower (g)**	**Numbers of floret/ spike**	**Weight of spike (g)**	**Stalk length (cm)**	**Spike length (cm)**	**Length of individual flower (cm)**	**Weight of individual flower (g)**	**Numbers of floret/ spike**	**Weight of spike (g)**
2nd week old plant	20.42	-	-	-	-	-	48.68	-	-	-	-	-
3rd week old plant	31.07	7.47	1.01	0.39	21.98	8.57	63.40	20.82	2.12	1.38	30.19	41.66
4th week old plant	47.53	9.46	1.23	0.45	25.13	11.31	73.42	20.63	4.56	2.19	36.45	79.83
5th week old plant	49.30	11.39	1.18	0.51	22.10	11.27	96.62	31.62	5.05	3.16	33.12	104.66
6th week plod plant	46.80	13.51	1.21	0.43	22.21	9.55	103.03	35.77	4.49	2.45	37.56	92.02

Table 2: Comparison between the healthy and foliar nematode infested flower stalk and the flower production attributes during 2013 and 2014 ratoon crops.

Months	**Healthy Flower Stalk**						**Infested Flower Stalk**					
	Stalk length (cm)	**Spike length (cm)**	**Length of individual flower (cm)**	**Weight of individual flower (g)**	**Numbers of floret/spike**	**Weight of spike (g)**	**Stalk length (cm)**	**Spike length (cm)**	**Length of individual flower (cm)**	**Weight of individual flower (g)**	**Numbers of floret/spike**	**Weight of spike (g)**
July'13	93.45	36.94	3.98	2.23	30.75	68.57	63.49	21.55	2.56	1.04	21.12	21.96
August'13	82.50	38.37	4.75	2.40	38.00	91.20	62.34	19.45	2.34	0.78	20.19	15.75
September'13	97.58	40.10	5.12	2.45	42.16	103.29	71.34	20.45	2.87	0.76	19.12	14.53
October'13	99.87	39.58	6.21	3.28	48.75	159.90	74.04	21.97	2.01	1.06	20.12	21.33
November'13	95.78	37.60	5.12	3.03	34.50	104.54	75.12	20.44	3.01	0.54	23.19	12.52
January'14	102.06	40.11	5.01	3.08	36.75	113.19	77.94	17.88	2.89	0.75	13.39	10.04
February'14	98.73	40.18	6.12	2.70	44.00	118.80	77.54	19.03	2.45	0.78	24.35	18.99

March'14	105.42	40.82	4.20	3.25	41.56	135.07	74.27	19.06	2.34	0.98	20.18	19.78
April '14	98.75	38.83	4.76	3.35	39.75	133.16	62.73	19.12	2.03	0.68	18.29	12.44
May '14	107.10	40.83	5.34	3.45	40.50	139.73	59.30	14.90	1.79	0.63	12.19	7.68
June'14	88.55	38.45	5.98	2.16	30.67	66.25	54.45	15.10	1.86	0.45	25.13	11.31
July'14	89.05	39.20	5.34	3.12	40.12	125.17	64.27	18.98	2.15	0.65	21.91	14.24
August'14	83.95	36.81	4.98	2.56	34.98	89.55	53.93	14.98	2.07	0.89	19.13	17.03
September'14	92.85	40.53	4.19	2.06	36.12	74.41	61.91	19.98	2.13	1.09	19.59	21.35
October'14	95.20	41.17	4.25	3.45	38.98	134.48	72.48	21.49	2.34	1.02	21.98	22.42
November'14	96.97	40.07	5.20	3.40	41.18	140.01	73.50	20.72	3.04	1.10	19.60	21.55

Table 3: Comparison between the healthy and foliar nematode infested flower stalk and the flower production attributes during 2015 and 2016 ratoon crops

Months	Healthy Flower Stalk						Infested Flower Stalk					
	Stalk length (cm)	Spike length (cm)	Length of individual flower (cm)	Weight of individual flower (g)	Numbers of floret/spike	Weight of spike (g)	Stalk length (cm)	Spike length (cm)	Length of individual flower (cm)	Weight of individual flower (g)	Numbers of floret/spike	Weight of spike (g)
January'15	99.63	43.87	4.85	2.67	34.34	91.69	77.00	21.00	2.10	1.12	18.29	20.48
February'15	100.00	39.21	4.90	2.85	37.37	106.50	74.27	20.54	1.67	1.10	23.73	26.10
March'15	104.75	42.42	5.40	3.13	30.11	94.25	56.34	16.48	1.96	0.95	16.79	15.95
April'15	95.13	39.63	4.45	2.23	40.57	90.47	47.77	17.25	2.22	0.95	21.32	20.25
May'15	97.61	40.57	4.75	2.17	32.58	70.70	51.51	16.51	2.02	0.65	16.19	10.52
June'15	101.68	39.25	4.95	2.56	30.13	77.13	59.24	17.74	1.09	0.73	19.09	13.94
July'15	98.87	39.30	5.15	3.21	42.19	135.43	57.30	16.87	2.07	0.78	22.10	17.23
August'15	102.23	41.13	6.01	3.65	42.34	154.54	46.11	17.54	1.60	0.96	20.74	19.91
September'15	99.21	40.03	4.45	3.01	28.11	84.61	55.55	17.05	1.59	0.79	24.46	19.32
October'15	100.44	40.57	4.15	4.10	31.26	128.17	63.73	21.08	2.03	1.05	19.11	20.06
November'15	100.45	41.50	6.12	3.10	42.10	130.51	62.55	21.05	2.58	1.13	20.30	22.93
January'16	97.43	40.92	4.56	3.50	31.89	111.61	72.10	18.32	2.56	1.07	21.09	22.57
February'16	98.68	41.08	3.95	3.31	32.56	107.76	69.78	17.11	2.04	1.12	21.70	24.30
March'16	101.39	39.53	4.05	3.08	36.45	112.27	48.12	12.49	1.86	0.97	14.87	14.42
April '16	99.28	40.43	5.05	3.21	32.33	103.79	45.98	14.66	2.13	0.88	20.59	18.10
May '16	93.95	36.30	4.40	2.80	37.56	105.17	50.56	18.16	2.13	0.76	18.65	14.20
June'16	86.15	32.00	4.75	2.50	37.01	92.53	55.45	16.39	2.01	0.82	18.71	15.37
July '16	87.10	28.50	3.65	2.98	40.23	119.89	53.91	14.45	1.87	0.91	16.16	14.62

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