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A review on nematodes and their management in tuberose

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Abstract
Tuberose (*Polianthes tuberosa* L.) is one of the most important flowers used both as cut and loose flower. It is extensively cultivated in many sub-tropical and tropical parts of the world including India. It is a crop which flowers profusely throughout the year. Due to the longer keeping quality of flower spikes, they are in great demand for making floral arrangement and bouquets. However, the yield loss is huge due to several problems which is a serious cause of concern. Nematode are the thread like worms, predominantly survives in plant rhizosphere, posing threat to tuberose cultivation. Nematodes *viz*., root-knot nematode (*Meloidogyne incognita* and *M. javanica*) and reniform nematode (*Rotylenchulus reniformis*) and greasy streak caused by *Aphelencoides besseyi* have been reported to be responsible for complete wiping out of tuberose flower industry. The symptoms of nematode infestation in broadly categorized as above ground and below ground symptoms. The management strategies include, hot water treatment of bulbs, use of neem cake, use of neem seed kernel extract, use of bio-control agents, nematicide application, genotype screening, mutation induction *etc* are recommended scientifically. In this review paper the magnitude of nematode problems, symptoms and management measures are discussed in a holistic manner.

Keywords: tuberose, plant parasitic nematodes, symptoms, management strategies and mutation

Introduction
Tuberose is an important half hardy bulbous perennial plant known for its colour, purity, elegance and fragrance of flowers. It is one of the major commercial flower crops, popularly known for various uses *viz*., decoration of floral ornaments, perfumes, beautification of home gardens and as cut flower. Currently it is grown in many countries of the world such as Vietnam, China, Brazil, Italy, Iran, UK, USA etc. including India (Kadam *et al.*, 2019) [16, 17]. Botanically, it is known as *Polianthes tuberosa*, belongs to the family of Amaryllidaceae. It is native of Mexico. It is a bulbous perennial plant with tuberous roots producing long spikes, bearing waxy white fragrant flowers which impregnate the atmosphere with their sweet fragrance. It is a crop which flowers profusely throughout the year. Owing to its longer keeping quality of flower spikes they are in great demand for making floral arrangement and bouquets in many parts of the world. Based on number of whorls of petals (corolla segments), the tuberose is classified into three categories, namely single, semi-double and double. The single type does have only one row of corolla segments, semi- double type bear flowers of two to three rows of corolla segments and double type has more than three rows of corolla segments.

The spikes as a whole in double types can be used as cut flowers whereas the florets of single varieties are used for making garlands, veni, gajra, bangles, etc. and also for essential oil (fragrance principle) extraction. The flower yields a very valuable floral concrete (aroma embedded in plant wax) upon solvent extraction. Generally, the recovery of concrete ranges from 0.08 to 0.11 per cent (Singh, 1995). The absolute of tuberose is the purest form of fragrance is extracted from floral concrete and is used in the preparation of various high value perfumes and cosmetics. The flower oil contains methyl benzoate, methyl anthranilate, benzyl alcohol, butyric acid, eugenol, nerol, farnesol and geraniol.

Despite its economic and agronomic significance, cultivation of tuberose in challenged by many biotic and abiotic factors. The biotic factors include insects (thrips, aphids), diseases (basal rot, stem rot, bulb rot) and nematodes. The abiotic factors pose threat to tuberose are water stress (drought), salt stress (salinity) and water stagnation (inundation). Among these factors, tuberose cultivation is increasingly being threatened by nematodes. The nematodes that infest plants including tuberose are generally called as plant parasitic nematode (PPN) and is one of the most limiting factor with respect to yield and quality of flowers. Different nematodes are infecting tuberose, causing yield and economic loss. The nematodes *viz.*, root-knot nematode (*Meloidogyne incognita* and *Meloidogyne javanica*) and reniform nematode (*Rotylenchulus reniformis*) and greasy streak caused by *Aphelencoides besseyi* have been reported to be responsible for complete wiping out of tuberose flower industry. Among the PPN, the most devastating are Root Knot Nematode (RKN) and Foliar Nematode (Saha and Khan, 2016) [37].

Root-knot nematode
Root-knot nematode (*Meloidogyne incognita*) has been reported as one of the important limiting factors affecting commercial cultivation of tuberose (Sunderbabu and Vadivelu, 1988) []; reducer of flower yield up to 10% (Khan and Parvatha Reddy,

1992) [37]. The infection of root knot nematode makes the plants highly susceptible to infection by *Fusarium oxysporum* f.sp. *dianthi* (Rao *et al*., 2003) [33]. Infestation of nematode symptoms is characteristics; affected crop shows chlorotic foliage, general stunting, below ground portion with heavy root galling (Johnson, 1970). Root knot nematode problem in tuberose is widespread; majority of the tuberose fields in North and South India are heavily damaged by the nematode (Rao *et al*., 2001) [34]. Root knot nematode infests root system of tuberose and often severely destroys the roots in association with other soil-borne fungi (Saha and Khan, 2016) [37]. Root knot nematode infects tissues of bulb and root and the yield loss was reported upto 10%. Therefore, using bulb as a planting material potentially disseminates root knot nematode from one place to another. The level of nematode inoculum of *M. incognita* is directly related to the damage on tuberose (Chawla *et al.* 2006) [7]. Most of the root knot nematodes (58.35%) were present up to a depth of 5.2 mm, in tuberose bulbs. No nematodes were observed at a depth of > 13.0 mm as reported by Gowda and Chawla, (2013) [11].

Symptoms of root knot nematode (Meloidogyne incognita and Meloidogyne javanica)

Infection of root-knot nematode produces characteristic disease symptoms on the below ground root system popularly known as 'root gall' or 'knotted roots'. The size of galls also differs with the level of infection. In the case of heavy infection, large size or multiple galls or secondary galls develop. Above ground symptoms are non-specific in nature. Infected plants exhibit symptoms of general mineral deficiency *viz*., yellowing, stunting, wilting during hotter part of the day, chlorosis, premature shedding of leaves and poor look of plants resulting in low yield. The nematodes are also involved in interaction with other soil borne fungi, bacteria, and viruses and cause serious damage to crops. (Khan, 2012) [21]. Chawla *et al*, (2008) [6]. observed the presence of egg mass of root knot nematode in bulb of tuberose and the nematodes were able to survive and multiply within the bulb tissues for 8 months in storage. Sellaperumal (2013) [39]. found nematode survived in the harvested bulbs for more than seven months and remain active within bulb. Nematode lifecycle continued in stored bulbs and these were the source of inoculum. The storage moisture content of bulbs played critical role in survival of root knot nematode. A moisture content of the bulbs during storage was more than 60%, was found to be sufficient for nematode survival, development and multiplication within bulb. Tuberose bulbs procured from root-knot nematode infested field, when planted in pots even containing sterilized soil, showed the symptoms of root-knot nematode infection (Chawla *et al.*, 2006) [7]. Sellaperumal *et al*,. (2013) [39]. reported that in tuberose bulbs, root-knot nematode laid eggs in gelatinous matrix, however, egg masses were completely embedded in the bulb tissues. No egg masses were observed on the outer surface of tuberose.

Symptoms of reniform nematode (Rotylenchulus reniformis)

Symptoms of damage to crops can be divided into two categories *viz*., above ground symptoms and below ground symptoms. Generally, the symptoms are non-specific both on the above ground and below ground parts. This necessities a close observation to confirm the presence of nematode and its damage. Reniform nematode feeds on cortical tissue, phloem and pericycles and its infection causes root necrosis. Symptoms appear as root discolouration, shedding of the leaves and formation of malformed flowers. In addition to causing direct damage to plant roots, the nematode in association with other pathogens such as *Fusarium* spp., *Verticillium* spp., *Sclerotium rolfsii* and *Rhizoctonia solani* develop disease complexes. It has also been reported to parasitize the bacterial nodules (Khan,, 2012) [21]. Saha and Khan, 2016 [37] studied the soil inhabiting plant parasitic nematodes, *R. reniformis* was encountered in high densities in West Bengal while *Helicotylenchus* spp., and *H. indicus* were estimated in low densities from tuberose rhizosphere.

Symptoms of foliar nematode (*Aphelenchoides besseyi)*

In tuberose (*Polianthes tuberosa* L.), the foliar nematode was first discovered to induce foliar disease in Hawaii (Holtzmann, 1968). The occurrence of 'floral malady' caused by *A. besseyi* in tuberose was found in the Ranaghat areas of the Nadia district of West Bengal in India (Chakraborti and Ghosh, 1993) [4].

Fig 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 D: Spiny outgrowth (prickle) or rugged surface on the scape of the nematode heavily infested tuberose crops (cv. Calcutta double) reported by Kadam *et al.*, 2019 [16, 17].

Fig 2: 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) reported by Kadam *et al.*, 2019 [16, 17].

Infected flower stalk initially appears rough, stalk becomes crinkled, stunted and finally distorted and in severe cases flower buds fail to bloom. Brown streaks appear on leaf bracts and petals and subsequently develop rusty brown spots. The severely infected flower stalk becomes rotten and brittle over drying, even get blind. The number of flowers per stalk is also reduced and small crinkled and distorted flowers are produced which are not acceptable in the market. The nematode, *A. besseyi* remains in masses forming 'nematode wool' which could be easily recovered from dark brown spots (Khan, 1999 [26]; Khan and Pal, 2001) [24]. Chakrabarti and Ghosh (1993). recorded a floral malady problem in tuberose from Ranaghat area of Nadia, West Bengal. Tuberose foliar nematode (*A. besseyi*) inducing floral malady in Calcutta double and Calcutta single cultivars is one of the limiting factors for cultivation of tuberose in West Bengal, India.

Khan (2004) [19]. recorded that A. besseyi survived in all the stages under anhydrobiotic condition by coiling its body at room temperature (15-35^{O}C) for more than 25 months. The nematode could survive in the slow drying scaly leaves, floral parts and stalks but not in soil even for one crop season. Its dissemination occurred through planting bulbs, flower stalks, scaly leaves and floral parts and stalks. Foliar nematode affects tuberose plants at various stages causing reduction in plant vigor, yield, quality and marketability of flowers (Pathak and Khan, 2009) [32]. The nematode infestation started at young flower primordial stage and causing flower malady symptom in upcoming tuberose stalk and causing flower malady in tuberose and huge amount of revenue loss (Grewal and Jagdale, 2001) [12]. Bala (2007) [2]. observed that the *Aphelenchoides besseyi* reached the peak during July in Calcutta double cultivar. Kadam *et al.,* (2019) [16, 17]. studied tha correlation between population dynamics of nematode and weather parameters. They revealed that in *cv.* Calcutta Double, *A. besseyi* maintained the least population during December to February when average temperature, total rainfall and relative humidity remained quite low.

Fig 3: Comparison between healthy and foliar nematode infested flower (cv. Calcutta single)

Fig 4: Comparison between healthy flower stalk and blind head (cv. Calcutta single)

Fig 5: Comparison between healthy flower stalk and foliar nematode infested stalk Reported by Bala (2007) [2].

Field diagnosis of nematode problem

A. Above ground field symptoms

a. Stunting growth, reduction of foliage and progressive decline of plants
b. Yellowing (chlorosis) of leaves
c. Leaf with angular spots and necrosis, white tip
d. Wilting of plant in hot sunny days
e. Early senescence
f. Poor yield
g. Reduction in number of spike
h. Deformed or distorted foliage (crinkling, curling and twisting), flowers (floral malady)
i. Formation of gall

B. Below ground symptoms

a. Root knot or root gall, swelling of root tip, forking, branching or stubbed/ abbreviated root
b. Root lesions or brown/black spots, root discolouration
c. Lesion or deformation on tubers, rhizomes etc.
d. Dirty root with soil adhered to surface (Jain *et al.,* 2010 and Khan. 2012) [21].

Management strategies

1. Root Knot nematode

- Tsang *et al.,* 2001 [41] found that hot water treatment has been used to disinfest planting materials from plant-parasitic nematodes.
- The biocontrol potential of fungal bio agents particularly using *Paecilomyces lilacinus* and *Trichoderma harzianum* either alone or in combination with neem cake and FYM against *Meloidogyne incognita* have been tested and often more than one applications have been suggested (Rao, 2007) [33]. for assured nematode management.
- The growth promoting rhizobacteria (*Pseudomonas fluorescens*) suppressed infestation of *M. incognita* (Haq *et al*., 2011).
- Sultan *et al*. (2011) reported in tuberose that application of nematicide, Carbofuran and recorded increased bulb yield despite having significant root galling due to *M. incognita* in plots treated with neem cake, neem seed powder, *P. lilacinus* and *Trichoderma harzianum*.
- Saha and Khan (2016) [37]. revealed that the application of bioformulation of *Paecilomyces lilacinus* @ 5 kg enriched with FYM 5t/ha was found to be the most effective and economic for reduction of root knot nematode infestation, nematode population and enhancement of cut-flower yield of tuberose.
- Nagesh and Krishna (2004) [30]. evaluated the effect of bulb treatment with a combination of nematode antagonist *Pochoni chlamydosporia*, plant growth promoting fungus, *Glomus mosseae* along with neem cake (500 kg/ha) were effective against root knot nematode in tuberose. Bulbs treated with a combination of neem cake amendment plus *P. chlamydosporia* and *Glomus mosseae* observed maximum percent of healthy root, flower spike/m^2, spike length and no. of florets/spike, least number of root gall and nematode population in soil and root.
- The application of *Trichoderma harzianum* @ 5kg with FYM 5t/ha was also comparable to Carbofuran @ 1 kg a.i./ha for root knot nematode management in tuberose (Saha and Khan, 2016) [37].
- In tuberose 27 germplasm of both single and double tuberose were screened for their tolerance / resistance to *M. incognita.* Among them, Suvarna Rekha recorded the least gall index and found to be resistant, followed by Calcutta Double, Bidhan Rajani-3, Variegated, Arka Shringar, IIHR-12 and Bidhan Rajani-2 which had lower gall indices and revealed tolerance towards *M. incognita*. Whereas rest of the genotypes had higher gall index and categorized under susceptible. In the tolerant genotype, the giant cell formation was disrupted with higher number of phenolic cells and cells with lignified cell wall that prevented the successful establishment of nematode feeding site. The resistant and tolerant varieties recorded significantly higher accumulation of phenol as well as polyphenol oxidase (PPO), peroxidase (PO) and phenyl alanine ammonia lyase (PAL) activities compared to the susceptible varieties. Nutritional changes in nematode infected leaves was observed and existed a significant difference in nutrient content among nematode infected germplasm. Higher nutrient content in nematode resistant, tolerant genotypes than susceptible genotypes was reported by Gandhi, 2007 [8].

2. Reniform nematode

- Gautam *et al.* (2005) [10]. screened ten cultivars of tuberose (*Polianthes tuberosa* L.) *viz*., Sikkim Selection, Prajwal, Shringar, Rajat Rekha, Mexican Single, Hyderabad Single, Pearl Double, Vaibhav, Swarna Rekha and Suvasini against reniform nematode (*Rotylenchulus reniformis*). Nematode multiplied in good numbers on all the tested cultivars. Nematode number on roots was the least on cultivar Swarna Rekha. Maximum number of nematodes on root as well as in soil were observed on cultivar Pearl Double. Multiplication factor (initial nematode population/final nematode population) of more than 6 was observed on all the tested cultivars of tuberose, The higher multiplication factor indicated absence of resistance in tested tuberose cultivars to reniform nematode. Further, a non-significant difference was observed between the multiplication factor values of Single Petalled and Double Petalled type cultivars, which is an indicative of equal susceptibility in both the types of
- cultivars. Application of nematicide either Thimet or Furadan (20 kg/ha), Furadon @ 2 g/plant or Carbofuran @ 2-5 kg/ha, neem cake @ 1 tonne/ha control nematode infestation (Safeena *et al.,* 2015) [36].

3. Foliar nematode

- Pre-soaking of bulbs for overnight followed by hot water treatment at 50^0C for 20 min (Khan *et al*., 2005 and 2006) [22, 23] or dipping of bulbs in Monocrotophos 36SL at 750 ppm for 6 h (Khan *et al*., 2008) [25]. Khan *et al.* (2005) [22]. observed that cent percent mortality of *A. besseyi* was obtained at 48 ± 2^{O}C with exposure of five minutes but germination of tuberose bulb was not affected with hot water treatment at 50 ± 2^{o}C for 30 minutes. Khan *et al.,* (2005) [22]. found that pre-soaking of bulbs for overnight followed by Hot Water Treatment at 50^0C for 30 min + dipping of bulbs in Monocrotophos 36 SL in 0.05% for 6 h + two sprayings with Monocrotophos 36 SL at 0.05% in first year crop and

three sprayings with Monocrotophos 36 SL at 0.05% at 15 days interval in the second and third year crop was superior in terms of suppression of nematode population and production of quality flowers.

- Pre-soak of infected tuberose bulbs in plain water for over-night followed by hot water treatment. Result of the experiment showed that overnight pre-soaking of infected bulbs in plain water followed by hot water treatment at 53^0C for 60 minutes was found the most efficacious to reduce nematode population in the first year crop as well as first ratoon crop and also enhanced growth of tuberose. The post stalk nematode population in the stalk was always higher than the bulbs of the same plant which act as potential source of inoculam in tuberose (Bala, 2016) [1].
- Three to four sprayings with Monocrotophos 36SL at 500 ppm at 15-20 days interval starting at bulb sprouting.
- During peak flowering stage additional spraying with Neem Seed Kernel Extract (NSKE) 5% at 15 days interval.
- Infested plant parts should be burnt immediately on inspection.
- Growing nematode tolerant cultivars.
- Run-off or irrigation water from infested field should not be allowed to enter the healthy, nematode free tuberose fields (Khan, 2006b) [23].
- Chakraborti and Dutta (1997) [5]. also found efficacy of dipping of bulbs in azadirachtin, garlic, clove extract along with neem cake as soil amendment in reduction of floral malady in tuberose.
- Chakraborti (1995) also found the efficacy of Monocrotophos at 0.15% against *A. besseyi* in tuberose.
- Pal *et al.,* (2013). reported to control of nematodes can be obtained by manipulating the dates to avoid periods of peak nematode activity and prevent reduction in yield. Growing nematode resistant cultivar Prajwal give the grower an assured yield of tuberose flower. The closer spacing increases the nematode infection providing congenial environment to nematode, thus wider recommended spacing is followed. Judicious use of nematicides can protect the tuberose from nematode infection without undesirable effects on the environment. Here the use of non-fumigant
- nematicide- Carbosulfan resulted in 7.45% increase in yield in the second year of planting over the non-treated ones.

Development of resistance through induction of Mutants in tuberose

In tuberose, irradiation at lower dose of 10 and 20 kR gamma rays the population of nematode was less in both M_1 and M_2 generation and at 10 + 10 kR in recurrent M_2 generation. Whereas in Ethyl methane Sulfonate (EMS) treatments, the population of nematodes was less at 20 and 30 mM in both M_2 generation and 20+20 mM in recurrent M_2 generation. The similar trend was observed in per cent of wilted plants in the field (Dhivya, 2015). The experiments conducted by Kayal*viz*hi (2016) [18]. revealed that both physical (gamma ray) and chemical mutagen (EMS & DES) were ineffective in inducing nematode resistant mutants in tuberose cv. Prajwal. The observations on root knot nematode and soil nematode population indicated that all the treated plants recorded a higher gall index of '5' and thus categorized under 'highly susceptible' in tuberose.

Conclusion

Tuberose is an important commercial flower crop in India and many parts of the world and is cultivated for its fragrance and market potential. The root-knot nematodes (*Meloidogyne incognita* and *Meloidogyne javanica*), reniform nematode (*Rotylenchulus renioformis*) and greasy streak nematode (*Aphelencoides besseyi*) are reported to cause major damage to the crop, which is characterized by the stunted growth of the plants and resulting in extensive yield losses. The leaf size is reduced and the flowers look sickly and ultimately, the roots start to decay. The above discussed management strategies are useful to research scholars, scientists and farmers.

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