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Management of tomato root-knot wilt complex caused by *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *lycopersici* through plant extracts

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Abstract
Efficacy of different plant extracts were evaluated against root-knot wilt disease complex of tomato caused by *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *lycopersici*. Plant extracts were used both as seed treatment and seedling treatment with 2% concentration. Among tested plant extracts, NSKE was found most effective that reduces nematode population (No of Galls/ plant 20.68 & No of Egg masses/ plant 15.67) and wilt disease incidence 21.64 followed by garlic clove, it also significantly increase plant growth parameters (shoot length 53.07cm, root length 19.37cm, shoot weight 29.63g fresh & 7.30g dry and root weight 7.61g fresh & 2.25g dry).

Keywords: Plant extracts, tomato, wilt, *Meloidogyne incognita*, *Fusarium oxysporum*

Introduction
Tomato (*Solanum lycopersicum* Mill.) is the second most important vegetable belongs to the family *Solanaceae*. Tomato crop suffers from various phytopathogenic diseases. Among the fungal diseases, Wilt disease caused by *Fusarium oxysporum* f. sp. *lycopersici* [1] is most important disease and is limiting factor in tomato production. The fungus also develops synergistic relationship with *Meloidogyne* spp. leading to root-knot wilt disease complex [2]. The first recorded case of a nematode–fungus interaction was made by Atkinson, 1892 [3], who observed that fusarium wilt of cotton (caused by *Fusarium oxysporum* f. sp. *vasinfectum*) was more severe in the presence of root- knot nematodes (*Meloidogyne* spp.). This complex is highly destructive to tomato plants and is characterized by enhanced wilt symptoms [4]. Root knot infection causes 24-26% loss in tomato [5]. A yield loss of 35 - 39.7% has been reported due to root-knot nematode infestation on tomato and estimated percentage of loss of 39.77 at 20 larvae /g soil in tomato field in Karnataka [6]. Various management strategies have been used extensively over the years to manage the diseases but the use of plant extracts as bio-pesticides for nematode and fungus disease complex is most economic and environment friendly method. So, the use of plant extracts in Integrated Pest Management (IPM) is now accepted as an ecologically sound alternative to chemical pest control. Thus, the present experiment was conducted to management of the wilt disease complex caused by *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *lycopersici* in tomato using plant extracts as non-chemical approaches.

Materials and Methods
Preparation and maintenance of pure culture of *Fusarium oxysporum*
Diseased samples were collected from farmer's field of the tomato growing areas of Jaipur district and brought to the laboratory of Department of Plant Pathology, S.K.N. College of Agriculture, Jobner for isolation and further examination.
Tomato roots were washed and then cut into small pieces. These pieces were surface sterilized in 1 per cent sodium hypochlorite solution for 1-1½ minute followed by three consecutive washing with sterilized distilled water, the pieces were transfer to potato dextrose agar medium in petri plates and incubated at 25+1 ºC in BOD incubator for 7 days. The fungal colonies emanating from bits were examined on 7 days of incubation. Pure culture of the fungus was obtained by Single Spore Technique.

The fungus was multiplied on pre-soaked sterilized sorghum grain in flask by inoculating with 7 days old culture of *Fusarium oxysporum* f. sp. *lycopersici* and incubated at 25±1 °C temperature for 7 days.

Preparation and maintenance of pure culture of *Meloidogyne incognita*

Root knot nematode infested tomato plants were collected from the fields of Jaipur district and nearby vegetable growing villages and brought to the laboratory. Egg masses were carefully detached from roots, under a stereoscopic binocular microscope. Egg masses, collected from the infected roots were kept in distilled water in watch glasses at room temperature for hatching. Freshly hatched 2nd stage juveniles were then inoculated on one month old tomato seedlings which were grown and maintained in 20cm radius clay pots filled with steam sterilized soil to obtained adequate pure population of *M. incognita* on the plants and in soil to carry out further experiments.

Preparation of plant extract

Neem seed kernel extract, giloy stem, garlic clove, lantana leaves, aak leaves and tulsi leaves were collected and washed with distilled water. Hundred gram of clean fresh plant parts were ground with 100 ml absolute alcohol. The mixture was allowed to stand for 48 hours at room temperature and subsequently filtered through filter paper. The solvent was completely evaporated from the extract at 70º C till it become a semi-solid material. This semi-solid material becomes stock solution by adding distilled water and makes different respective concentration by different plant extracts stock solution.

***In-vivo* effect of plant extracts on the *Fusarium oxysporum* f. sp. *lycopersici* and *Meloidogyne incognita* infecting tomato**

Two per cent concentration was prepared with 2 ml of stock solution and 98 ml of distilled water. Seven tomato seedlings were dipped in six different plant extracts and sown in pots. Distilled water alone serves as control {Nematode at the time of sowing and fungus one week after (N1+F2)}. Tomato seedlings of uniform size were dipped in plant extracts. The treated seedlings were transplanted in pot. The experiment was laid out in CRD with five replications. The pots were watered regularly as and when required. Observation on plant growth, reproduction of root-knot nematode and wilt per cent disease incidence on tomato were recorded at 45 days after transplanting. The roots were washed carefully under tap water and stained with 0.1 per cent acid Fuschin lactophenol and after wash kept in clear lacto phenol for 24 hrs. Thereafter the roots were examined thoroughly under a stereoscopic binocular microscope for counting number of galls per plant and number of egg masses per plant. The data were subjected to statistical analysis. Total number of treatments was 07 (T_1-NSKE, T_2-Giloy stem, T_3-Garlic clove, T_4-Lantana leaves, T_5-Aak leaves, T_6-Tulsi leaves and T_7-Control).

Results and Discussion

(A) Growth Parameter

Shoot Length

The data indicated that maximum shoot length was recorded with NSKE (53.07 cm) followed by garlic cloves (50.10 cm), Aak leaves (48.23 cm). Rest of the treatment were observed to be inferior to NSKE but significantly superior over control (21.33 cm).

Root Length

The data indicated that maximum root length was recorded with NSKE (19.37 cm) followed by garlic cloves (18.03 cm) and Aak leaves (16.87 cm) over control (8.27cm).

Shoot Weight

The maximum shoot weight was recorded with NSKE (29.63 g fresh and 7.30 g dry) followed by garlic clove (27.78 g and 6.68 g) and aak leaves (26.41 g and 5.53 g). While, minimum recorded in control (4.01 g and 0.54 g).

Root Weight

The maximum root weight was recorded with NSKE (7.61 g fresh and 2.25 g dry) followed by garlic clove (7.01 g and 1.99 g) and aak leaves (6.59 g and 1.80 g).

(B) Nematode Reproduction

No. of Root Galls

Number of galls were significantly reduced in NSKE (20.68) as compared to control where maximum (41.27) galls were recorded, followed by Garlic clove recorded (22.35) and aak leaves (25.11). Rest of the treatments were inferior over these but superior over control.

No. of Egg Masses

Number of egg masses were significantly and drastically reduced in NSKE (15.67) as compared to control where maximum (32.67) egg masses were recorded.

(C) Wild Incidence

Percent disease incidence

Among all the treatments minimum per cent disease incidence of wilt was recorded with NSKE (21.64%) followed by garlic clove (23.01%) and Aak leaves (26.56%). All the treatments significantly reduce wilt disease incidence of wilt as compared to control (61.37%).

Table 1: Efficacy of Plant extracts (botanicals) on tomato plant growth parameters

Treatments	Shoot Length (cm)	Root Length (cm)	Shoot weight (g)		Root weight (g)	
			Fresh	Dry	Fresh	Dry
NSKE	53.07	19.37	29.63	7.30	7.61	2.25
Giloy stem	37.23	12.43	19.16	4.06	4.94	1.17
Garlic clove	50.10	18.03	27.78	6.68	7.01	1.99
Lantana leaves	46.30	15.73	23.78	4.97	5.73	1.61
Aak leaves	48.23	16.87	26.41	5.53	6.59	1.80
Tulsi leaves	42.03	14.77	21.47	4.48	5.37	1.38
Control (N1+F2)	21.33	8.27	4.01	0.54	0.74	0.52
S.Em%	0.653	0.768	0.636	0.328	0.483	0.166
CD at 5%	1.957	2.303	1.905	0.985	1.447	0.498

*Average of five replications

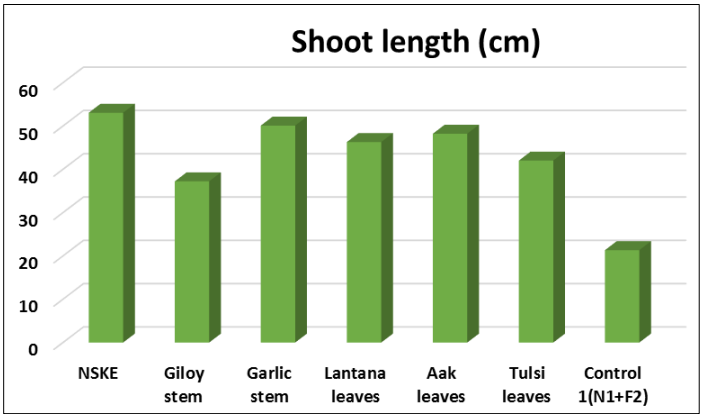
NSKE = Neem Seed Kernel Extract

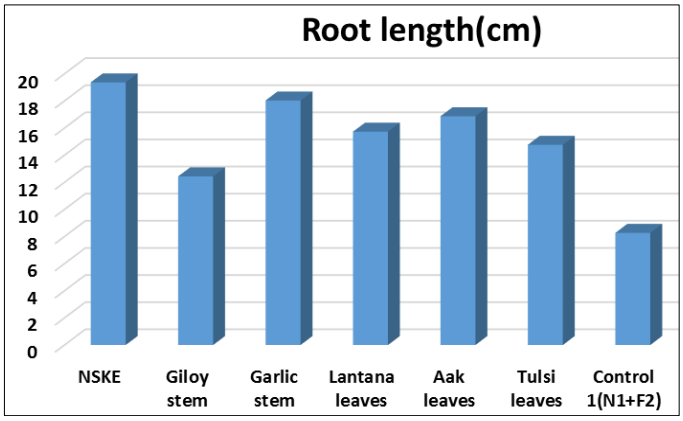
Table 2: Efficacy of plant extracts (botanicals) on wilt (*Fusarium oxysporum* f. sp. *lycopersici*) incidence and *Meloidogyne incognita* interaction in tomato

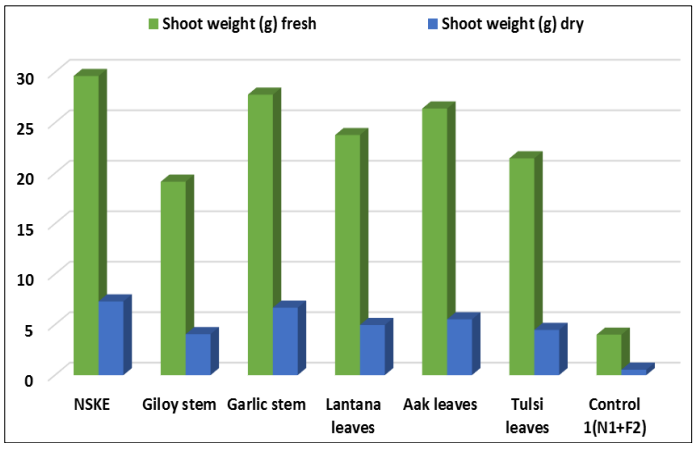
Treatments	No of Galls/ plant	No of Egg masses/ plant	PDI
NSKE	20.68	15.67	21.64 (27.72)
Giloy stem	34.02	26.00	42.9 (40.92)
Garlic clove	22.35	18.00	23.01 (28.66)
Lantana leaves	28.69	21.00	32.97 (35.04)
Aak leaves	25.11	19.32	26.56 (31.02)
Tulsi leaves	30.54	23.67	40.37 (39.44)
Control (N1+F2)	41.27	32.67	61.37 (51.57)
SEm%	0.198	1.037	0.893
CD at 5%	0.592	3.108	2.677

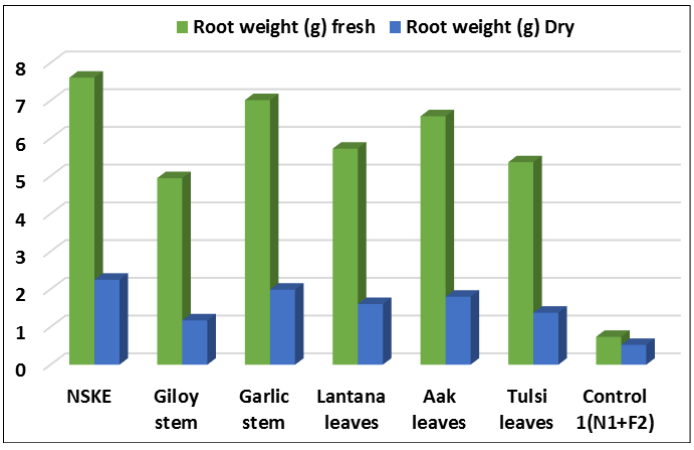
*Average of five replications
Figures given in parenthesis are angular transformed value
NSKE = Neem Seed Kernel Extract

The present investigation revealed that among different plant extracts (NSKE, Giloy stem, Garlic clove, Lantana leaves, Aak leaves and Tulsi leaves), NSKE found most effective followed by garlic cloves and aak leaves in increase in plant growth parameters (shoot length, root length, shoot weight and root weight) and reduction in number of galls, egg masses and per cent disease incidence. Similar results were reported on tomato [7] and green gram [8]. Kumar and Khanna, 2006 [9] reported significant and improved plant growth by effectively reducing nematode population by applying NSKE followed by Eco neem. Neem products were more toxic (NSKE as compare to NSE) to *Meloidogyne incognita* than to *Cephalobus persegnis* and followed by *Heterorhabditis indica* [10]. Similarly, garlic cloves extract found effective in reduction of number of galls, egg masses on roots and juveniles of *M. incognita* in soil. Similar results were reported by Youssef *et al*., 2016 [11]. Similarly, Aak leaves were effective in reduction of the soil nematode population {Saravanapriya and Sivakumar, 2005 [12] and Patel *et al*., 1993 [13]}. Application of neem cake significantly decrease the root-knot nematode population on tomato [14], neem leaves powder [15, 16], *Calotropis procera* and *Aloe vera* [17] was found to be the most effective in improving growth of maize and reducing infection of *Heterodera zeae*.









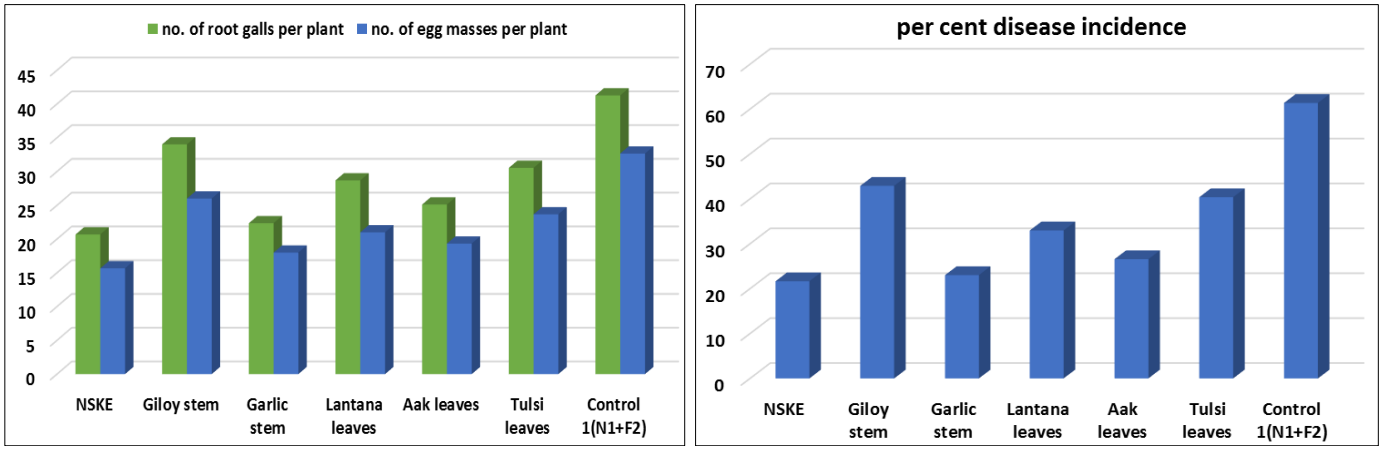


Fig 1: Efficacy of various plant extracts (botanicals) on wilt (*Fusarium oxysporum* f. sp. *lycopersici*) incidence and *Meloidogyne incognita* in tomato under pot condition

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