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Nematode-fungi interaction on growth parameter and biochemical changes on tomato

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Abstract
Nematode -fungi interaction on growth parameters and severity of disease on tomato revealed that among the different inoculation combination, nematode inoculate prior to fungus (N→F) was most destructive, followed by simultaneously inoculation of fungus and nematode (F+N). In alone treatment, *P. aphanidermatum* was found most damaging and reduced the length by 32 and 28%, fresh weight 69 and 76% and dry weight 82 and 60% of shoot and root, respectively over control. Whereas in the sequential treatments, where fungal pathogen was inoculated 3 days before nematode inoculation (F→N), all pathogens significantly decreased the plant growth parameters and they were at par with the alone /single inoculation treatment. Among the all treatments, *F. o.* f. sp. *lycopersici* with *M. incognita* was recorded most destructive treatment and reduced the length 50 and 32%, fresh weight 72 and 76%, and dry weight 86 and 61% of shoot and root, respectively over control. Among the simultaneously inoculated treatments (F+N), combination of *P. aphanidermatum* + *M. incognita* was recorded most destructive and reduced the number of flower / plant (75%) and weight of fruits/ plant (72 %) compared to un- inoculated control plant. Biochemical assay of total phenol and salicylic acid contents also revealed that increase concentrations of both the chemicals were found in inoculated plants than the un-inoculated plants. In alone treatment, inoculation of *P. aphanidermatum* caused maximum increased in total phenolic contents (60%,) compared to un-inoculated control. Among the simultaneous treatments, combination of *P. aphanidermatum* + *M. incognita* was increased of total phenolic contents by 52%, followed by *R, solani*+ *M. Incognita* (47%) compared to un- inoculated control. Similarly, the maximum increased per cent of salicylic acid was recorded in *P. aphanidermatum* with *M. incognita as* 24% in compared to un-inoculated control. *F. oxysporum* f. sp. *lycopersici* with *M. incognita* treatment caused least increase in Salicylic acid content (14%). Among the simultaneous treatments (F+N) combination of *P. aphanidermatum* + *M. incognita* was recorded highest deceased in Salicylic acid content (37%) followed by *F. oxysporum* f. sp. *lycopersici* +*M. incognita* decreased Salicylic acid content (28%) in compared to un- inoculated control.

Keywords: nematodes-fungi interaction, growth parameters, simultaneous inoculation, phenol content

Introduction
Tomato (*Solanum lycopersicum* L.) is the second most important vegetable crop after potato in the world. The annual production of tomato in the world is about 177 million tonnes (FAO, 2016). The crop is cultivated as an annual crop in most region of the world (Vossen *et al.*, 2004) [17]. India ranks fourth in the world in tomato production after China, United States and Turkey with an estimated production of 22 million tonnes in 2016-17 from about 7.3 per cent of the entire cropped land (Anonymous, 2016-17) [1]. In India, Andhra Pradesh is the leading state in both area and production which contributes 18% of the total production of tomato in India, followed by Arunachal Pradesh, Assam and Bihar. The production of tomato is influenced by a number of factors which includes edaphic and environmental factors as well as pests and pathogens. Soil-borne pathogens, especially, *Rhizoctonia, Fusarium* and *Pythium* are highly destructive and cause tremendous yield loss to tomato crop. Root-rot caused by *R. solani*, is one of the dreaded diseases, prevalent throughout the tomato growing regions around the world and causes moderate to severe damage to the crop. The severely infected roots may lead to complete failure of the crop. Tomato wilt caused by *F. oxysporum* f. sp. *lycopersici* occurs almost in every tomato growing area of world including India and other countries and causes up to 15% annual loss (Mandal *et al*., 2009) [10].
Plant diseases of complex etiology is not simple as of specific etiology because it involves two or more pathogens and the disease is governed by the action and activity of the engaged two or

more pathogens and the relationship that developed between them. In this context, the present study was under taken to investigate the interaction of *Meloidogyne incognita* with *Pythium aphanidermatum*, *Rhizoctoniasolani*, and *Fusarium oxysporum* f. sp. *lycopersici* in order to determine their damaging potential on tomato cv. Local, and to also understand the effect of single, sequential and concomitant inoculations of the pathogen with root-knot nematode on plant growth, yield parameters, salicylic acid and total phenol contents of leaf of tomato.

Material and Method

Nursery culture of tomato

The seed of tomato cv. *Local* was procured from local market of Aligarh, The nursery was raised in earthen pots (30x30 cm) in the month of February. The pots were filled with 5 kg mixture of soil, sand and farm yard manure (3:1:1), and autoclaved at 15kg/cm^2 pressureat 121ºC for 15-20 minutes. Tomato seeds were sown in the pot. Pot were kept on a cemented platform and watered as and when considered necessary.

Collection of root-knot nematode, *Meloidogyne incognita*

Infected root samples of tomato showing galls or knots were collected from cultivation unit in and around Aligarh. The root samples were collected in polythene bags and brought to the laboratory. Roots where rinsed under the slow stream of water thereafter females and egg masses from the galled tissue were excised. The species of *Meloidogyne incognita* (Kofoid and White) Chitwood was confirmed using perineal pattern technique of ten females from the each root system (Barker *et al.*, 1985) [2]. To prepare inoculation of nematode, egg masses of *M. incognita* infected rootswere excised from the root samples and placed on course sieve lined with two layer of tissue paper which was then put in a Baermann's funnel filled with adequate amount of water. Most of the larvae hatched out from the egg masses and migrated across the tissue paper reaching in the stem of the funnel during incubation of one week at 25±2ºC. Nematode suspension from the stems was collected and standardized by counting number of larvae/ml suspension in a counting dish under stereomicroscope.

Collection and mass culture of fungal pathogens

Pure culture of *Rhizoctonia solani*, *Fusarium oxysporum* f. sp. *lycopersici* and *Pythium aphanidrmatum* were procured from the ITCC, Division of Mycology and Plant Pathology, IARI, New Delhi. The pure culture was maintained on potato dextrose agar (PDA) in culture tubes and Petri plates (fig.1) and stored in a refrigerator at 5ºC. For mass culturing of *R. solani*, *F. o.* f. sp. *lycopersici* and *P. aphanidrmatum* the fungus was multiplied on sorghum grains. The seeds were soaked overnight in 5% sucrose and 0.0003% chloramphenicol solution (Whitehead, 1957) [18]. The seeds were transferred to conical flasks of 500 ml capacity and autoclaved twice at 15 kg/cm^2 pressure at 121ºC for 15-20 minutes. Thereafter, flasks were inoculated with the pure culture of *R. solani*, *F. o.* f. sp. *lycopersici* and *P. aphanidrmatum* as separately. After inoculation, the flasks were incubated for 10 days in a BOD incubator at 25±2ºC. The contaminated flask, if any was removed immediately and discarded. During incubation, the flasks were shaken daily manually for a few minutes to promote uniform colonization on seeds. The incubation period, if required, was extended until the entire medium was fully colonized by fungus.

Inoculum level and doses

Sorghum seeds colonized by *R. solani*, *F. o.* f. spp. *Lycopersici* and *P. aphanidrmatum* were weighed macerated in the distilled water in an electric grinder to make fungus suspension. The suspension containing 2g colonized seeds (2 x 10^6) was applied to soil in each pot (1 kg soil)

Treatments detailed.

Earthen pots of 15 cm diameter were filled with 1 kg mixture of soil, sand and farm yard manure (3:1:1) and were autoclaved at 15 kg/cm^2 at 121.ºC for 15-20 minutes. Following 13 treatments were maintained.

T_1 = Plant alone (Control)
T_2 = *Rhizoctoniasolani* alone
T_3 = *Fusarium oxysporum* f. sp. *lycopersici*alone
T_4 = *pythiumaphanidrmatum* alone
T_5 = *M. incognita* alone
T_6 = *R. solani* → *M. incognita*
T_7 = *F. oxysporum* f. spp. *Lycopersici* → *M. incognita*
T_8 = *P. aphanidrmatum* → *M. incognita*
T_9 = *R. solani* + Nematode
T_{10} = *F. oxysporum* f. spp. *lycopersici* + Nematode
T_{11} = *P. aphanidrmatum*+*M. incognita*
T_{12} = *M. incognita* → *R. solani*
T_{13} = *M. incognita* → *F. oxysporum* f. spp. *lycopersici*
T_{14} = *M. incognita* → *P. aphanidrmatum*

Where
R→N = *Rhizoctonia* applied 3 day before the nematode
F→N = *Fusarium* applied 3 day before the nematode
P→N = Pythium applied 3 day before the nematode
R+N= *Rhizoctonia* & nematode applied simultaneously
F+N= *Pythium* applied 3 day before the nematode
P+N= *Pythium* & nematode applied simultaneously
N→R= Nematode applied 3 day before the *Rhizoctonia*
N→F= Nematode applied 3 day before the *Fusarium*
N→P = Nematode applied 3 day before the *Pythium*

Three to four leaved old seedlings of tomato (local variety) were transplanted at the center of a pot after application of above treatments and/or inoculation with pathogens. Three pots (replication) of each treatment were maintained. Seedlings were watered immediately after planting. Plants were grown for four months. During this period, they were regularly observed for any visible symptom attributable to pathogens At harvest (4 month after planting), pots were flooded with water to achieve maximum root recovery and following parameters were determined

i) Length of root and shoot
ii) Fresh weight of root and shoot
iii) Dry weight of root and shoot
iv) Estimation of total phenol contents
v) Estimation of salicylic acid (SA)
vi) Number of flower and fruit of plant
vii) Total weight of fruit/ plant

Biochemical changes

Estimation of total phenol contents

Leaf samples (1g) from tomato plants 10 days after inoculation were homogenized in 10 ml 80% methanol and agitated for 15 minutes at 70°C (Zieslin and Ben Zaken, 1993). Leaf from the three plants of a treatment was processed separately. One milliliter of the methanol extract was added to 5 ml of distilled water and 250 µl of Folin-Ciocalteau reagent (1N) and solution was kept at 25°C. The absorbance of the developed blue color was measured using a

Spectrophotometer (Spectronic 20, USA) at 765nm. Gallic acid was used as the standard. The amounts of phenolics were expressed as gallic acid mg/g fresh weight of leaf sample.

Estimation of salicylic acid (SA)

Salicylic acid of the leaves of the treatment was estimated separately 10 after inoculation. The leaves were cut into small pieces of size 0.5-1.0 cm, soaked in water for overnight, than filtered through the Watman filter paper no.1 and extracted in ethyl acetate. The ethyl acetate fraction was taken and sodium sulphate added to remove the moisture and filtrate was evaporated to dryness in water bath. The stock solution was prepared by the addition of 10 ml methanol. The stock solution was used for recording the absorbance in a spectrophotometer (SHIMADZU, 2450 PC, Japan) at 306 nm. The absorbance was fixed at 306 nm and the readings were recorded at different ppm of SA and standard curve was prepared for the estimation of SA concentration in the leaf sample (Pankaj *et al.,* 2005) [12]. The readings were taken at 306 nm absorbance. The readings were recorded at different concentration of SA (ppm). The standard curve was plotted to get a best fit line passing through the origin. From the standard curve the concentration of SA in the sample was calculated according to the formula y = mx ± c (Lowery *et al*., 1951) [8].

Statistical Analysis

Observations taken from three pots were averaged to calculate means. The data (3 replicates/ treatment) on the plant growth, yield parameter, total phenol content and salicylic acid were analyzed by analysis of variance (ANOVA) and least significance difference (LSD) was calculated at a probability level of data $P \leq 0.05$, to identify significant effect of a treatment (Dospekhov, 1984). Galls and egg masses index, root rot index, wilt index and final soil population of fungus and nematode was presented as figure using MS excel-2010. Percent variation over control was also calculated.

Results and Discussion

Plant growth parameters

The data present in the table showed that inoculation of *R. solani, F. oxysporum* f. sp. *lycopersici, P. Aphanidermatum* and *M. incognita* in alone caused significant decline in plant growth parameters. However, among the pathogens, *P. aphanidermatum* was found most damaging and reduced the length by 32 and 28%, fresh weight 69 and 76% and dry weight 82 and 60% of shoot and root, respectively over control (Table 1). Next destructive pathogen was *F. oxysporum* f. sp. *lycopersici*, which reduced the length by 30 and 26%, fresh weight 68 and 70%, and dry weight 81 and 58% of shoot and root, respectively over control (Table 1). *R. solani* alone also significantly reduced the lengths 31 and 27%, fresh weight 70 and 63% and dry weights 79 and 53% of shoot and root, respectively over control. Alone inoculation of second stage juveniles of *M. incognita* least damaging and reduced the lengths 24 and 26%, fresh weight 68 and 63% and dry weight 78 and 44% of shoot and root, respectively over control.

In the sequential treatments, where fungal pathogen was inoculated 3 days before nematode inoculation (F→N), all pathogens significantly decreased the plant growth parameters and they were at par with the alone /single inoculation treatment. Among the treatments, *F. o.* f. sp. *lycopersici* with *M. incognita* was recorded most destructive treatment and reduced the length 50 and 32%, fresh weight 72 and 76%, and dry weight 86 and 61% of shoot and root, respectively over control (Table 1). The treatment of *P. aphanidermatum* with *M. incognita* which reduced the length 50 and 25%, fresh weight 71 and 76%, and dry weight 80 and 59% of shoot and root of tomato, respectively over control, followed by *R. Solani* which reduced the length 45 and 23%, fresh weight 70 and 66%, and dry weight 83 and 61% of shoot and root, respectively over control (Table 1).

In other sequential treatments, where *M. incognita* J_2 was inoculated 3 day before fungal pathogen, (N→F), all pathogens significantly decrease the plant growth parameters. Among the treatments, inoculation of *M. incognita* with *P. aphanidermatum* was found highest destructive and reduced the length 61 and 37%, fresh weight 76 and 77%, and dry weight 85 and 63% of shoot and root of tomato, respectively over control (Table 1). The next destructive pathogen as *F. o.* f. sp. *lycopersici* with *M. incognita,* which reduced the length 56 and 35%, fresh weight 75 and 74%, and dry weight 82 and 62% of shoot and root respectively over control. Treatment of *R. solani* with *M. incognita* was found least destructive and reduced the length 50 and 28%, fresh weight 74 and 74%, and dry weight 81 and 52% of shoot and root of tomato, respectively over control (Table 1).

The combine treatment of fungal pathogens and nematode (F+N) leads to synergistic relationship between them. It has found that symptom were more pronounced and appeared earlier than the N→F and alone treatments. Among the simultaneous treatments, combination of *P. aphanidermatum + M. incognita* was recorded highly destructive and reduced the length 60 and 29%, fresh weight 72 and 66%, and dry weight 85 and 65% of shoot and root, respectively over control (Table 1). Simultaneously treatment of *R, solani*+ *M. incognita*, which reduced the length 57 and 23%, fresh weight 72 and 64%, and dry weight 79 and 55% of shoot and root, respectively over control. *F. o.* f. sp. *lycopersici + M. incognita* also significantly reduced the lengths 54 and 28%, fresh weight 72 and 66%, and dry weight 80 and 72.% of shoot and root, respectively over control (Table 1). Root-knot nematodes are important pest of solanaceous crops and induce significant reduction in growth and yield of many crop (Khan and Akram, 2000). Oluma and Oladiran, (1993) reported that the rotting disintegrates the internal tissue of the main root and impairs of the ability of roots to absorb water and minerals, as a results progressive decline of the aerial growth occurs and ultimately the growth and biomass of the plants are reduced as observed in the present investigation. They also found that higher loss due to wilt pathogen indicating that the fungus directly impaired vital physiological function such as absorption of water, minerals etc and chlorosis which led to subsequent decrease in the dry matter production as occurred in the study. Khan and Dasgupta, (1993) [6] found that role of root-knot nematode as predisposing agents in fungus-nematode disease complex has been well established they found that nematode promotes the pathogenesis of secondary pathogen, however, it may leads to the suppressive effect on the nematode themselves, as observed in present study. However, the combinations of the two pathogens leading to significant reduction plant growth parameters indicating the synergistic interaction in comparison to reduction caused by two pathogens individually. Other researchers have also reported the synergistic interaction of *M. incognita* with *F. oxysporum* f. sp. *lycopersici* (Kumar *et al*., 2017), *P. aphanidermatum* (Jasim *et al*., 2013) and *R. solani* (Husain *et al*. 1985) and present study is consistent with previous reports on tomato.

Table 1: Effect of inoculation of *Meloidogyne incognita, Rhizoctonia solani*, *Pythium aphanidermatum* and *Fusarium oxysporum f. sp. lycopersici* alone and different combination on plant growth parameters of tomato plant

Treatment	Length (cm.)		Fresh weight (g)		Dry Weight (g)	
	Shoot	Root	Shoot	Root	Shoot	Root
Control (Un-inoculated)	43.66	11.50	18.16	6.83	7.66	2.50
Rhizoctonia alone (R)	30.08 (-31.10)	8.40 (-26.95)	5.50 (-69.7)1	2.50 (-63.39)	1.63 (-78.72)	1.16 (-53.6)
Fusarium alone (F)	30.33 (-30.53)	8.45 (-26.52)	5.83 (-67.89)	2.06 (-69.83)	1.44 (-81.20)	0.99 (-58.0)
Pythium alone (P)	29.66 (-32.06)	8.32 (-27.65)	5.66-68.83)	1.63 (-76.13)	1.38 (-81.98)	1.00 (-60.0)
Nematode alone (N)	33.33 (-23.66)	8.50 (-26.08)	5.83 (-67.89)	2.49 (-63.54)	1.68 (-78.06)	1.38 (- 44.8)
R→N	23.83 (-45.41)	8.83 (-23.21)	5.40 (-70.26)	2.33 (-65.88)	1.27 (-83.72)	0.97 (-61.2)
F→N	21.70 (-50.29)	7.83 (-31.91)	5.00 (-72.46)	1.67 (-75.54)	1.06 (-86.16)	0.96 (-61.6)
P→N	22.00 (-49.61)	8.60 (-25.21)	5..25 (-71.09)	1.66 (-75.69)	1.46 (-80.93)	1.03 (-58.8)
R+N	18.60 (-57.39)	8.83 (-23.21)	5.15 (-72.46)	2.44 (-64.27)	1.62 (-78.85)	1.12 (-55.2)
F+N	20.00 (-54.19)	8.33 (-27.56)	5.03 (-72.30)	2.31 (-66.17)	1.55 (-79.76)	0.95 (-62.0)
P+N	17.83 (-59.61)	8.20 (-28.69)	5.00 (-72.46)	2.70 (-60.22)	1.18 (-84.59)	0.87 (-65.2)
N→R	21.60 (-50.52)	8.25 (-28.26)	4.70 (-74.11)	1.78 (-73.78)	1,40 (-8.72)	1.03 (-58.0)
N→F	19.16 (-56.11)	7.41 (-35.56)	4.40 (-75.27)	1.59 (-74.23)	1.35 (-82.37)	0.97 (-61.2)
N→P	17.16 (-60.69)	7.25 (-36.95)	4.30 (-76.32)	1.39 (-77.50)	1.16 (-84.85)	0.93 (-62.8)
LSD P ≤ 0.05	2.12	0.91	0.82	0.57	0.41	0.27

Values are means of three replicates. Values in parenthesis are percent variation over control.

Where;

R→N = *R. solani* applied 3 days before the *M. incognita*

P→N = *P. aphanidermatum* applied 3 day before the *M. incognita*

F→N = *F. oxysporum* f. sp. *lycopersici* applied 3 days before the *M.*

R+N = *R. solani* and *M. incognita* applied simultaneously

F+N = *F. oxysporum* f. sp. *lycopersici* and *M. incognita* applied simultaneously

P+N = *P. aphanidermatum* and *M. incognita* applied simultaneously

N→R = *M.* incognita applied 3 days before the *R. solani*

N→F = *M. incognita applied 3 days before the F. oxysporum* f. sp. *lycopersici*

N→P = *M. incognita applied 3 days before the P. aphanidermatum*

Plant Yield

Combine inoculation with *R. solani*, *F. o.*f sp. *lycopersici*, *P. aphanidermatum* and *M. incognita* alone and in different combinations caused significant decline in plant yield of tomato and reduced total number of flowers and total weight of fruit/ plant but they did not affect the number of fruit / plant. In alone treatment inoculation of *P. aphanidermatum* caused highest reduction in yield parameter compared to un-inoculated control. *P. aphanidermatum* reduced the number of flower / plant (66%), weight of fruit / plant (59%, Table 2). The pathogen, *F. o.*f. sp. *lycopersici* which reduced the number of flower / plant (46%) and weight of fruit / plant (55%) followed by *R. Solani* which reduced the number of flower / plant (52%) and weight of fruits / plants (52%), compared to un- inoculated control plant (Table 2). *M. incognita* caused minimum loss to the yield parameter and reduced the number of flower / plant (36%) and weight of fruit / plant (45%) compared to un- inoculated control plant.

Among the sequential treatment (F→N), inoculation of *R. solani, F. o.*f sp. *lycopersici, P. Aphanidermatum* before 3 day of *M. incognita,* combination of *F. o.*f. sp. *lycopersici* with *M. incognita* was found most destructive and reduced the number of flowers / plant (64%) and weight of fruits / plant (70%) compared to un- inoculated control plant (Table 2). Next destructive pathogen was *P. aphanidermatum* with *M. incognita,* which reduced the number of flowers / plant (55%) and weight of fruit / plant (61%) compared to un- inoculated control plant. *R. Solani* with *M. incognita* was found least destructive and reduced the number of flowers / plant (44%) and weight of fruits / plant (54%) compared to un- inoculated control plant (Table 2).

The combine treatment of (N→F), i.e., inoculation of *M. incognita* before 3 days of *R. solani, F. o.* f sp. *lycopersici* and *P. aphanidermatum* combination of *P. aphanidermatum* with *M. incognita,* was found most destructive and reduced the number of flower / plant (57%) and weight of fruits / plant (82%) compared to un- inoculated control plant (Table 2). Next destructive pathogen was *F. o.* f. sp. *lycopersici* with *M. incognita,* which reduced the number of flower / plant (53%), weight of fruit / plant (59%), followed by *R. Solani* with *M. incognita* and reduced the number of flower / plant (54%) and weight of fruit / plant (77%) compared to un- inoculated control plant (Table 2).

Among the simultaneously inoculated treatments (F+N), combination of *P. aphanidermatum* + *M. incognita* was recorded most destructive and reduced the number of flower / plant (75%) and weight of fruits/ plant (72 %) compared to un- inoculated control plant (Table -2). Next destructive pathogen was *R. solani*+ *M. incognita,* which reduced the number of flowers / plant (55%), weight of fruit / plant (69%), followed by *F. o.* f. sp. *lycopersici* + *M. incognita,* which reduced number of flower / plant (75%), weight of fruit / plant (63%) compared to un- inoculated control plant (Table -2). This indicates that the nematode accelerate the pathogenesis of root-rot and wilt fungus leading to significant reduction in yield. The interaction was synergistic as the combined treatment caused higher reduction in tomato yield than the two pathogens individually. Singh and Mathurr (2010a) have reported 24-61% decrease in the yield of tomato due the root-knot infestation. Similarly, 15-62% (Ramamoorthy *et al*., 2002) [13], 12-15% (Mandal *et al*. 2009) [10], and 10-80% (Hadwan and Khara, 1992) yield loss due to *P. aphanidermatum, R. solani* and *F. o.* f. sp. *lycopersici,* respectively, have been recorded in other studies.

Table 2: Effect of inoculation of *Meloidogyne incognita, Rhizoctoniasolani*, *Pythium aphanidermatum* and *Fusarium oxysporum f. sp. Lycopersici* alone and different combination on yield parameters of tomato plant.

Treatment	Number of flower/ plant	Number of fruit per plant
Control (Un-inoculated)	9	9
Rhizoctonia alone (R)	4.3 (52.3%)	4.3 (52.3%)
Fusarium alone (F)	4.8 (46.66%)	4.8 (46.66%)
Pythium alone (P)	3 (66,66%)	3 (66,66%)
Nematode alone (N)	5.7 (36.43%)	5.7 (36.43%)
R→N	5 (44.32%)	5 (44.32%)
F→N	3.2 (64.23%)	3.2 (64.23%)
P→N	4 (56.34%)	4. (56.34%)
R+N	4.1 (54..44%)	4.1 (54..44%)
F+N	4.2 (53.37%)	4.2 (53.37%)
P+N	3.8 (57.77%)	3.8 (57.77%)
N→R	4 (55.22%)	4 (55.22%)
N→F	3.4 (62.30%)	3.4 (62.30%)
N→P	2.2 (75.55%)	2.2 (75.55%)
LSD P ≤ 0.05	0.48	0.21

Values are means of three replicates. Values in parenthesis are percent variation over control.
Where;
R→N = *R. solani* applied 3 days before the *M. incognita*
P→N = *P. aphanidermatum* applied 3 day before the *M. incognita*
F→N = *F. oxysporum* f. sp. *lycopersici* applied 3 days before the *M.*
R+N = *R. solani* and *M. incognita* applied simultaneously
F+N = *F. oxysporum* f. sp. *lycopersici* and *M. incognita* applied simultaneously
P+N = *P. aphanidermatum* and *M. incognita* applied simultaneously
N→R = *M.* incognita applied 3 days before the *R. solani*
N→F = *M. incognita applied 3 days before the F. oxysporum* f. sp. *lycopersici*
N→P = *M. incognita applied 3 days before the P. aphanidermatum*

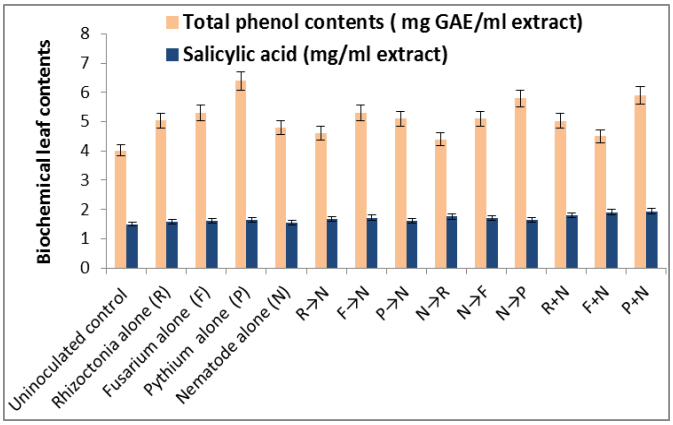
Biochemical changes

Total phenolic compound

The data present on the table showed that at 10 days after planting total phenolic contents of tomato leaves was recorded from all the treatment, which varied greatly concentration of total phenolic contents was observed in un-inoculated plant (Fig. 1). However, in pathogen inoculated plant, total phenolic contents significantly increased but it varied on with the treatments. In alone treatment, inoculation of *P. aphanidermatum* caused maximum increased in total phenolic contents (60%,) compared to un-inoculated control. Which was followed by Inoculation of *R. solani* (46%), *M. incognita* total phenolic contents (39%). *F. oxysporum* f. sp. *Lycopersici* caused minimum increase in total phenolic contents (33%) compared to un-inoculated control.

In the sequential treatment, where fungal pathogen was inoculated 3 days before inoculation of nematode (F→N), all pathogens significantly increased the phenolic content but highest increased was observed in inoculation of *P. aphanidermatum* with *M. incognita* treatment (58%) compared to control (Fig. 1). Next pathogen with respect to total phenolic contents increase was *F. oxysporum* f. sp. *lycopersici* with *M. incognita*, which increased total phenolic contents content about 47%, followed by *R. solani* with *M. incognita*, which increased the total phenolic contents content 29% compared to un- inoculated control (Fig. 1).

In other sequential treatments, where *M. incognita* J_2 was inoculated 3 day before of fungal pathogen (N→F), all treatment significantly increased the total phenolic contents compared to un- inoculated control. In the N→F treatments, inoculation of *M. incognita* with *P. aphanidermatum* caused highest increased in total phenolic contents (45%) compared to un- inoculated control (Fig. 1. The next treatment with regarded to total phenolic contents *F. oxysporum* f. sp. *lycopersici* with *M. incognita* and it increased the total phenolic contents by 41%, followed by *R. solani* with *M. incognita* treatment (31%) compared to un- inoculated control (Fig. 1). Among the simultaneous treatments, combination of *P. aphanidermatum* + *M. incognita* was recorded highest increased of total phenolic contents (52%), followed by *R, solani*+ *M. incognita*, which also significantly increased the total phenolic contents (47%) compared to un- inoculated control (Fig. 1). *F. o.* f. sp. *lycopersici* +*M. incognita* treatment caused least increase in total phenolic contents (16%) compared to un- inoculated control (Fig. 1).



Where; R→N = *R. solani* applied 3 days before the *M. incognita*
P→N = *P. aphanidermatum* applied 3 day before the *M. incognita*
F→N = *F. oxysporum* f. sp. *lycopersici* applied 3 days before the *M.*
R+N = *R. solani* and *M. incognita* applied simultaneously
F+N = *F. oxysporum* f. sp. *lycopersici* and *M. incognita* applied simultaneously
P+N = *P. aphanidermatum* and *M. incognita* applied simultaneously
N→R = *M.* incognita applied 3 days before the *R. solani*
N→F = *M. incognita applied 3 days before the F. oxysporum* f. sp. *lycopersici*
N→P = *M. incognita applied 3 days before the P. aphanidermatum*

Fig 1: Effect of inoculation of *Meloidogyne incognita, Rhizoctoniasolani*, *Pythium aphanidermatum* and *Fusarium oxysporum f. sp. Lycopersici* alone and different combination on total phenol and salicylic acid contents of tomato leaves 10 days after inoculation. Bars shows standard error.

Among all the combinations, alone treatment caused minimum increase in total phenolic contents, followed by N→F and F→N. The maximum increased in total phenolic contents was recorded when fungus and nematode inoculated simultaneously (F+N). Total phenol contents of tomato leaves significantly increased in fungus and nematode inoculated plants than the un-inoculated plants. This indicates that the phenolic compounds contribute in the defense of plants against pathogen attack (Nicholson and Hammerschmidt, 1992; Hammond Kosack and Jones, 1996) [11]. The phenolic contents of the concomitantly inoculated plants were much greater than the plants inoculated with either of the pathogen and joint effects of the two pathogens in relation to phenolic contents were more or less additive. Phenolic compounds are considered important in imparting host resistance and also inducing systemic acquired resistance in plants (Kuc, 1995) [7]. Greater synthesis of phenolic compounds in the plants inoculated with plant pathogenic fungi (Kuc, 1995) [7] and nematodes (Sirohi *et al.*, 2008) [16] has also been reported earlier as observed in the present study.

Salicylic acid

Salicylic acid content of tomato leaves was recorded at 10 days after planting and trend was similar to total phenolic contents. The lowest concentration of salicylic acid was recorded in un-inoculated plant (Fig. 1). However, inoculation of fungal pathogen and nematode significantly increased the Salicylic acid content irrespective of the treatments and combination. In alone treatments, inoculation *P. aphanidermatum* greatly increased Salicylic acid content (37%) in compared to un- inoculated control (Fig. 1). Next pathogen with respect to Salicylic acid content increased was *F. oxysporum* f. sp. *lycopersici* (31%) in compared to un-inoculated control. *R. solani* also significantly increased Salicylic acid content (14), followed by *M. incognita*, (11%) in compared to un- inoculated control (Fig. 1).

In the sequential treatment, F→N, all pathogens significantly increased the Salicylic acid content. The maximum increased was recorded in *P. aphanidermatum* with *M. incognita* Salicylic acid content (24%) in compared to un- inoculated control (Fig. 1). *F. o.* f. sp. *lycopersici* with *M. incognita* was found next in increase Salicylic acid content (12%), followed by *R. Solani* with *M. incognita*, which increased the Salicylic acid content by 5% in compared to un- inoculated control (Fig. 1). In other sequential treatments, where N→F, inoculation of *M. incognita* with *P. Aphanidermatum* caused highest increased in Salicylic acid content (21%), followed by *R. solani* with *M. incognita* (16%) in compared to un-inoculated control. *F. oxysporum* f. sp. *lycopersici* with *M. incognita* treatment caused least increase in Salicylic acid content (14%, Fig. 1). Among the simultaneous treatments (F+N) combination of *P. aphanidermatum* + *M. incognita* was recorded highest deceased in SAC (37%) followed by *F. oxysporum* f. sp. *lycopersici* +*M. incognita* decreased Salicylic acid content (28%) in compared to un- inoculated control (Fig. 1). *R, solani*+ *M. incognita* also significantly decreased salicylic acid content (16%) compared to un inoculated control.

Among all the combinations, simultaneously inoculation of fungus and nematode (F+N) caused maximum increased Salicylic acid content in tomato, followed by nematode inoculate prior to fungus (N→F). (F→N), and treatment fungus inoculate prior to nematode the minimum increased Salicylic acid content was recorded in alone treatment. Salicylic acid content has also been implicated as one of the key components in the signal transduction pathway leading to plant resistance to various pathogens was reported by several workers (Ryals *et al*., 1996., Wobbe and Klessig, 1996) [14]. Galls induced by nematode in inoculated plants is also accompanied by the accumulation of Salicylic acid content at the root site as well as in the phloem fluid and in healthy uninoculated leaves (Malamy *et al*., 1990; Enyedi *et al*., 1992; Summermatter *et al*., 1995) [9]. Greater accumulation of Salicylic acid content was also observed in tobacco leaves, due to infection caused by the fungi *Phytophthora parasifica*, *Cercospora nicotianae*, and *Peronospora tabacina* has been reported by Ryals *et al.*, (1996) [14].

It has been concluded from the present study that among the different inoculation combination, nematode inoculate prior to fungus (N→F) was most destructive treatment followed by simultaneously inoculation of fungus and nematode (F+N), Biochemical assay of total phenol and salicylic acid contents also revealed their higher concentration in inoculated plants than the un-inoculated plants. The concentration of total phenolic compounds and salicylic acid was much greater in the combined than the alone treatment.

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