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Bio-Efficacy of Phytotherapeutic Substances Against *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *cucumerinum* Affecting Cucumber in Polyhouse under Protected Cultivation

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ABSTRACT: The nematicidal effect of neem seed kernel extract (NSKE), neem, aak and castor leaves was tested against root-knot nematode, *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *cucumerinum*. Under the polyhouse conditions, all the treatments reduced the disease severity and enhanced the plant growth as compared to untreated inoculated check. Application of neem seed kernel extract (NSKE) was more effective as compared to neem leaves. Plant height, dry shoot weight, dry root weight, were significantly reduced as a results of infection with *M. incognita* and *F. oxysporum* f. sp. *cucumerinum*. However, application of phytotherapeutic substances recovered this reduction. Moreover, they enhanced the growth parameters viz., plant height, dry shoot weight, dry root weight compared to control. Our results proved that application of different phytotherapeutic substances, neem seed kernel extract (NSKE), neem, aak and castor leaves not only had a suppressive effect on the root knot nematode, but also enhanced the plant growth parameters, supplying nutritional elements to the plants. Among phytotherapeutic substances, *A. indica* seed kernel extracts were found to be most effective in suppressing galling and final population in soil and fungus wilt incidence after 30th day of germination followed by mustard and aak leaves as compared to untreated inoculated check.

Keywords: *Meloidogyne* spp., cucumber, neem seed kernel extract, neem leaves, aak leaves and castor leaves, fungus

Cucumber (*Cucumis sativus* L.) is widely cultivated crop in the gourd family Cucurbitaceae grown all over the world due to a good source of vitamins, minerals, fiber and roughages (Weng, 2011). Though in the polyhouses, crops are grown under protected conditions, yet they are not so protected. Polyhouse cultivation involves intensive cultivation of crops, optimum use of fertilizers and frequent use of irrigation. But due to continuous growing of the same crop with high day temperature and relative humidity within the greenhouse, polyhouse and low tunnel along with poor plant hygienic conditions inside and outside the greenhouse increase problem of soil borne pests and diseases including plant parasitic nematodes (Minuto *et al*., 2006) which results in the availability of ideal conditions for the growth and multiplication of these pests.

Under polyhouse cultivation, crops are attacked by a number of pests and diseases including nematodes which interfere with the successful cultivation under protected conditions ((Hanafi and Papasolomontos, 1999; Greco and Esmenjaud, 2004). Among the nematodes, root-knot nematode (*Meloidogyne* spp.) is the most damaging under polyhouse conditions, parasitizing almost all the polyhouses crops (Sharma *et al*., 2007). A frequency of occurrence of root-knot nematode under protected conditions was recorded to be 63.15% and population density range was 30-10000 j2/ 200cc soil (Patil *et al*. 2017). The damage becomes very severe in association with fungi (Abawi 1998). Though, yield loss due to this nematode is difficult to predict, approximate yield loss due to this nematode has been predicted by many authors in various crops (Kruger 2007). Another

important biotic stress to which the crop exposed is the fungus, *Fusarium oxysporum* f. sp. *cucumerinum.*

Generally, root knot nematode-fungus complex is considered to be one of the important factors responsible for the reduced crop production under field and polyhouse conditions. However, very little work has been done on the management of nematode-fungus disease complex in cucumber under polyhouses conditions. This research proposed to study the management nematode-fungus disease complex in cucumber under protected conditions by using different phytotherapeutic substances.

MATERIALS AND METHODS

Pure culture of root-knot nematode *Meloidogyne incognita* was maintained in the pots planted with tomato in the Nematology Laboratory. Fresh J2 were obtained from the eggs isolated from the infected roots and used in the experiment as required.

Pure culture of *F. oxysporum* f. sp. *cucumerinum*, isolated from the infested plants during random survey of polyhouses was maintained on PDA in petriplates at 27±1 °C. In order to produce mass-culturing pure culture of the fungus was grown on sand maize meal medium (700gm sand + maize meal 300gm + 150ml distilled water). The flasks and polypropylene bags were incubated in a BOD incubator at a temperature of 27±1°C for 15 days. During incubation, the flasks were shaken three times in a day, to ensure proper growth of the fungal mycelium on the sand maize meal medium.

Experimental procedure

Experiment was conducted in polyhouse (26.7±3°C, 73.5 % ± 11% Relative Humidity and 0.918 kPa) in earthen pots (18 cm diameter) filled with a mixture of autoclaved sandy loam soil (sand 70%, silt 22% and clay 8%, pH 7.5). Sterilized autoclaved soil was filled in pots (1 kg capacity). Autoclaved soil was subsequently infested with root-knot nematode (1000 J_2/kg soil) and fungus (50g /kg soil). Uninoculated pots and nematode + fungus inoculated pots served as controls. Chopped leaves of neem, aak, castor and neem seed kernel powder each @ 20 & 30 g/pot. Carbofuran 0.1 g/pot and Bavistin 2 g/1 water were used as chemical treatments. A waiting period of ten days was given before sowing for decomposition of leaves. Each pot was sown with cucumber cv. sania 5 seeds per pot. One plant per pot was retained after 30 days. The experiment was terminated 60 days after germination. Each treatment was replicated four times in a completely randomized block design during the months of April to June, 2015 in the polyhouse under protected conditions.

Evaluations were performed 60 days after sowing on plant growth parameters (shoot length, fresh and dry shoot and root weight). Observations were recorded on the root population, number of galls per plant, number of egg masses per plant, final nematode population per 200cc soil. Per cent wilt incidence due to fungus was also assessed. The data were subjected to one or two factorial Completely Randomized Design (CRD) using OPSTAT programme available on-line at CCS HAU, Hisar University website (www.hau.ernet.in).

RESULTS

Effect of different treatments on the plant growth parameters

The data (Table 1) indicated that shoot length in all the treatments were significantly better over untreated inoculated checks, nematode alone, fungus alone and nematode + fungus simultaneously. Among the various treatments, maximum shoot length was observed in neem seed kernel powder (NSKP) 30 g per kg soil, followed by neem leaves 30 g per kg soil irrespective of whether nematode or fungus inoculated individually or concomitantly. The data (Table 2) expressed that dry shoot weight in all the treatments was significantly better over untreated inoculated checks, nematode alone, fungus alone and nematode + fungus simultaneously. Among the various treatments, maximum dry shoot weight was observed in NSKP 30 g per kg soil, followed by neem leaves 30 g per kg soil irrespective of whether nematode or fungus inoculated individually or concomitantly. In plants inoculated with nematode and fungus concomitantly, dry shoot weight was maximum in case of NSKP followed by neem leaves as compared to untreated inoculated check.

Table 1. Effect of soil treatment with phytotherapeutic substances on shoot length (cm) of cucumber infested with *M. incognita* and fungus

Treatments	Shoot length (cm)			
	Nematode alone	Fungus alone	Nematode + fungus	Pooled Mean
T1: Neem leaves 20 g/ pot	133.3	128.8	126.1	129.4
T2: Neem leaves 30 g/ pot	151.9	147.1	145.9	148.3
T3: Aak leaves 20 g/ pot	128.6	124.1	122.3	125.0
T4: Aak leaves 30 g/ pot	143.5	139.5	136.7	139.9
T5: Castor leaves 20 g/ pot	123.0	119.4	116.5	119.6
T6: Castor leaves 30 g/ pot	138.9	134.8	132.2	135.3
T7: Soil treatment with NSKP 20 g/ pot	147.8	143.3	140.5	143.8
T8: Soil treatment with NSKP 30 g/ pot	157.3	153.9	150.9	154.0
T9: Carbofuran 0.1 g/ pot	143.4	121.6	140.1	135.0
T10: Drenching with bavistin 2 g/ l water	119.0	160.3	114.9	131.4
T11: untreated check (inoculated)	90.6	93.3	80.5	88.1
T12: untreated check (uninoculated)	162.5	164.8	165.0	164.1
Mean	136.6	135.9	131.0	

Critical Difference at 5% level, Treatment: 1.7, Sub treatment: 3.5, Treatment X Sub treatment: 6.1

Table 2. Effect of soil treatment with phytotherapeutic substances on dry shoot weight (g) of cucumber infested with *M. incognita* and fungus

Treatments	Dry shoot weight (g)			
	Nematode alone	Fungus alone	Nematode + fungus	Pooled Mean
T1: Neem leaves 20 g/ pot	14.06	13.18	12.74	13.3
T2: Neem leaves 30 g/ pot	21.37	18.98	17.25	19.2
T3: Aak leaves 20 g/ pot	13.25	12.01	11.11	12.1
T4: Aak leaves 30 g/ pot	16.83	15.75	14.75	15.8
T5: Castor leaves 20 g/ pot	12.25	10.97	10.25	11.2
T6: Castor leaves 30 g/ pot	15.33	14.75	13.57	14.5
T7: Soil treatment with NSKP 20 g/ pot	18.15	17.49	16.00	17.2
T8: Soil treatment with NSKP 30 g/ pot	24.24	22.34	21.75	22.8
T9: Carbofuran 0.1 g/ pot	19.50	12.25	18.49	16.7
T10: Drenching with bavistin 2 g/ l water	11.28	24.25	10.76	15.4
T11: untreated check (inoculated)	5.60	5.04	4.67	5.1
T12: untreated check (uninoculated)	25.24	26.01	25.00	25.4
Mean	16.4	16.1	14.7	

Critical Difference at 5% level, Treatment: 1.2, Sub treatment: 2.4, Treatment X Sub treatment: 4.3

The data (Table 3) revealed that dry root weight in all the treatments was significantly better over untreated inoculated checks, nematode alone, fungus alone and nematode + fungus simultaneously. Among the various treatments, maximum dry root weight was observed in NSKP 30 g per kg soil, followed by neem leaves 30 g per kg soil irrespective of whether nematode or fungus inoculated individually or concomitantly. However, in plants inoculated with nematode and fungus concomitantly, dry root weight was maximum in case of NSKP followed by neem leaves as compared to untreated inoculated check.

Effect of different treatments on the nematode reproduction

The data (Table 4) indicated that number of galls per plant in all the treatments was significantly reduced over untreated inoculated checks, nematode alone and nematode + fungus simultaneously. Among the various treatments, minimum number of galls per plant was observed in neem seed kernel powder (NSKP) 30 per kg soil (164), followed by neem leaves 30 g per kg soil irrespective of whether nematode inoculated individually or concomitantly. However, in plants inoculated with nematode and fungus concomitantly, number of galls per plant was minimum in case of NSKP, followed by neem leaves as compared to untreated inoculated check. Among the various treatments, minimum number of egg masses per plant was observed in NSKP 30 per kg soil, followed by neem leaves 30 g per kg soil irrespective of whether nematode inoculated individually or concomitantly. However, in plants inoculated with nematode and fungus concomitantly, number of egg masses per plant was minimum in case of NSKP, followed by neem leaves as compared to untreated inoculated check.

The data (Table 5) revealed that nematode population J_2/200 cc soil in all the treatments was significantly reduced over untreated inoculated checks *viz*., nematode alone and nematode + fungus simultaneously. Among the various treatments, minimum nematode population J_2/200 cc soil was observed in NSKP 30 per kg soil,

Table 3. Effect of soil treatment with phytotherapeutic substances on dry root weight (g) of cucumber infested with *M. incognita* and fungus

Treatments	Dry root weight (g)			
	Nematode alone	Fungus alone	Nematode + fungus	Pooled Mean
T1: Neem leaves 20 g/ pot	3.95	3.47	3.69	3.7
T2: Neem leaves 30 g/ pot	7.06	6.44	6.26	6.5
T3: Aak leaves 20 g/ pot	3.36	3.28	3.13	3.3
T4: Aak leaves 30 g/ pot	5.20	4.04	4.15	4.4
T5: Castor leaves 20 g/ pot	3.24	3.12	2.70	3.1
T6: Castor leaves 30 g/ pot	4.91	3.82	3.75	4.2
T7: Soil treatment with NSKP 20 g/ pot	5.52	4.28	4.44	4.8
T8: Soil treatment with NSKP 30 g/ pot	7.96	7.27	7.22	7.5
T9: Carbofuran 0.1 g/ pot	6.09	3.05	5.36	4.8
T10: Drenching with bavistin 2 g/ l water	3.07	5.75	3.27	4.1
T11: untreated check (inoculated)	1.75	2.24	1.34	1.2
T12: untreated check (uninoculated)	9.00	9.01	8.76	8.9
Mean	5.1	4.7	4.6	

Critical Difference at 5% level, Treatment: 0.37, Sub treatment: 0.75, Treatment X Sub treatment: 1.3

Table 4. Effect of soil treatment with phytotherapeutic substances on number of galls/ plant of cucumber infested with *M. incognita* and fungus

Treatments	Number of galls/plant		
	Nematode alone	Nematode + fungus	Pooled Mean
T1: Neem leaves 20 g/ pot	209 (14.5)	217 (14.7)	213 (14.6)
T2: Neem leaves 30 g/ pot	172 (13.2)	177 (13.3)	174.5 (13.2)
T3: Aak leaves 20 g/ pot	221 (14.9)	228 (15.1)	224.5 (15.0)
T4: Aak leaves 30 g/ pot	186 (13.7)	190 (13.8)	188 (13.7)
T5: Castor leaves 20 g/ pot	227 (15.2)	238 (15.4)	232.5 (15.3)
T6: Castor leaves 30 g/ pot	193 (13.9)	198 (14.1)	195.5 (14.0)
T7: Soil treatment with NSKP 20 g/ pot	177 (13.3)	183 (13.5)	180 (13.4)
T8: Soil treatment with NSKP 30 g/ pot	162 (12.8)	167 (12.9)	164.5 (12.9)
T9: Carbofuran 0.1 g/ pot	153 (12.4)	146 (12.1)	149.5 (12.3)
T10: Drenching with bavistin 2 g/ l water	230 (15.2)	215 (14.7)	222.5 (14.9)
T11: untreated check (inoculated)	328 (18.1)	313 (17.7)	320.5 (17.9)
T12: untreated check (uninoculated)	0 (1.0)	0 (1.0)	0.0 (1.0)
Mean	13.2	13.2	

Figures in parenthesis are the square root ("n+1) transformed values, Critical Difference at 5% level, Treatment: 0.03, Sub treatment: 0.07, Treatment X Sub treatment: 0.10

Table 5. Effect of soil treatment with phytotherapeutic substances on final nematode population/ 200 cc soil of cucumber infested with *M. incognita* and fungus

Treatments	Final nematode population/200 cc soil		
	Nematode alone	Nematode + fungus	Pooled Mean
T1: Neem leaves 20 g/ pot	295 (17.2)	300 (17.3)	297.5 (17.3)
T2: Neem leaves 30 g/ pot	261 (16.2)	267 (16.4)	264 (16.3)
T3: Aak leaves 20 g/ pot	312 (17.7)	319 (17.9)	315.5 (17.8)
T4: Aak leaves 30 g/ pot	276 (16.6)	281 (16.8)	278.5 (16.7)
T5: Castor leaves 20 g/ pot	317 (17.8)	327 (18.1)	322 (18.0)
T6: Castor leaves 30 g/ pot	282 (16.8)	288 (17.0)	285 (16.9)
T7: Soil treatment with NSKP 20 g/ pot	271 (16.5)	276 (16.6)	273.5 (16.6)
T8: Soil treatment with NSKP 30 g/ pot	248 (15.8)	252 (15.9)	250 (15.8)
T9: Carbofuran 0.1 g/ pot	167 (12.9)	235 (15.4)	201 (14.2)
T10: Drenching with bavistin 2 g/ l water	516 (22.7)	498 (22.3)	507 (22.5)
T11: untreated check (inoculated)	694 (26.4)	656 (25.6)	675 (26.0)
T12: untreated check (uninoculated)	0 (1.0)	0 (1.0)	0.0 (1.0)
Mean	16.5	16.7	

Figures in parenthesis are the square root ("n+1) transformed values, CD at 5% level, Treatment: 0.03, Sub treatment: 0.07, Treatment X Sub treatment: 0.1

followed by neem leaves 30 g per kg soil irrespective of whether nematode inoculated individually or concomitantly. However, in plants inoculated with nematode and fungus concomitantly, nematode population J_2/ 200 cc soil were minimum in case of NSKP, followed by neem leaves as compared to untreated inoculated check.

Effect of different treatments on the disease incidence

The data (Table 6) revealed that disease incidence of fungus was reduced significantly in all treatments on cucumber as compared to untreated inoculated checks. Data were recorded 15 and 30 days after sowing. At 30 days after sowing, disease incidence was minimum in case of soil treated with neem seed kernel powder (NSKP) followed by neem leaves as compared to untreated inoculated check while it was maximum in case of carbofuran. Data (Table 7) indicated that disease incidence of nematode and fungus complex was reduced significantly in all treatments on cucumber as compared to untreated inoculated checks. At 30 days after sowing, disease incidence was minimum in case of soil treated with NSKP followed by in case of neem leaves as compared to untreated inoculated check while it was maximum in case of carbofuran.

DISCUSSION

The present investigations showed that the phytotherapeutic substances were effective in reducing *M. incognita* infection, per cent disease incidence and increasing plant growth parameters as compared to untreated inoculated checks.

Shoot length in all the treatments was significantly better over untreated inoculated checks (nematode alone, fungus alone and nematode + fungus simultaneously). Maximum dry shoot weight was observed in treatments of neem seed kernel powder (NSKP) 30 g per kg soil and neem leaves 30 g per kg soil our findings are similar to the

Table 6. Effect of soil treatment with phytotherapeutic substances on fungal incidence (%) of cucumber infested with *F. oxysporum* f. sp. *cucumerinum*

Treatments	% Fungal incidence		
	Pre emergence damping off (after 15 days)	Pre emergence damping off (after 30 days)	Pooled Mean
T1: Neem leaves 20 g/ pot	35 (36.5)	40 (39.5)	37.5 (38.0)
T2: Neem leaves 30 g/ pot	20 (26.9)	25 (30.1)	22.5 (28.5)
T3: Aak leaves 20 g/ pot	35 (36.5)	40 (39.5)	37.5 (38.0)
T4: Aak leaves 30 g/ pot	25 (30.0)	30 (33.2)	27.5 (31.6)
T5: Castor leaves 20 g/ pot	40 (39.5)	45 (42.4)	42.5 (40.9)
T6: Castor leaves 30 g/ pot	30 (33.2)	35 (36.5)	32.5 (34.9)
T7: Soil treatment with NSKP 20 g/ pot	35 (36.5)	35 (36.5)	35 (36.5)
T8: Soil treatment with NSKP 30 g/ pot	15 (23.0)	20 (26.9)	17.5 (25.0)
T9: Carbofuran 0.1 g/ pot	40 (39.5)	45 (42.4)	42.5 (40.9)
T10: Drenching with bavistin 2 g/ l water	5 (13.6)	10 (18.6)	7.5 (16.1)
T11: untreated check (inoculated)	50 (45.3)	60 (51.1)	55 (48.2)
T12: untreated check (uninoculated)	0 (4.1)	0 (4.1)	0.0 (4.1)
Mean	30.4	33.4	

Figures in parenthesis are the angular transformed values, CD at 5% level: Treatment: 1.6, Sub treatment: 4.0, Treatment X Pathogen: N.S.

Table 7. Effect of soil treatment with phytotherapeutic substances on fungal incidence (%) of cucumber infested with *M. incognita* and fungus

Treatments	% Fungal incidence		
	Pre emergence damping off (after 15 days)	Pre emergence damping off (after 30 days)	Pooled Mean
T1: Neem leaves 20 g/ pot	30 (33.3)	40 (39.5)	35.0 (36.4)
T2: Neem leaves 30 g/ pot	20 (26.9)	25 (30.1)	22.5 (28.5)
T3: Aak leaves 20 g/ pot	30 (33.3)	40 (39.5)	35.0 (36.4)
T4: Aak leaves 30 g/ pot	25 (30.2)	30 (33.4)	27.5 (31.8)
T5: Castor leaves 20 g/ pot	35 (36.5)	45 (42.4)	40.0 (39.4)
T6: Castor leaves 30 g/ pot	25 (30.2)	30 (33.4)	27.5 (31.7)
T7: Soil treatment with NSKP 20 g/ pot	30 (33.4)	35 (36.5)	32.5 (35.0)
T8: Soil treatment with NSKP 30 g/ pot	15 (23.0)	20 (26.9)	17.5 (25.0)
T9: Carbofuran 0.1 g/ pot	15 (23.0)	10 (18.6)	12.5 (20.8)
T10: Drenching with bavistin 2 g/ l water	35 (36.5)	45 (42.4)	40.0 (39.4)
T11: untreated check (inoculated)	50 (45.3)	85 (67.7)	67.5 (56.5)
T12: untreated check (uninoculated)	0 (4.1)	0 (4.1)	0.0 (4.1)
Mean	29.6	34.5	

Figures in parenthesis are the angular transformed values, CD at 5% level: Treatment: 1.5, Sub treatment: 3.8, Treatment X Sub treatment: 5.4

results obtained by Agbenin *et al*. (2004) who reported that neem seed powder increased root/ shoot weights and heights but it reduces root knot nematode galls and presence of mycelium on root.

Number of galls per plant in all the treatments was significantly reduced over untreated inoculated checks, nematode alone and nematode + fungus simultaneously. Nematode population in soil in all the treatments was significantly reduced over untreated inoculated checks. Similar finding by Adegbite and Adesiyan (2005) who reported that neem seed reduces nematode population and it is effective in causing larval mortality. Also similar finding were reported by Agbenin and Marley (2004) that number of galls, egg masses and final nematode population in soil decreased significantly in the neem seed powder treated plants as compared to the untreated plants.

At 30 days after sowing, disease incidence was minimum in case of soil treated with neem seed kernel powder (NSKP) followed by neem leaves as compared to untreated inoculated check of fungus alone and these findings confirm that NSKP can be used as natural fungicides to control *F. oxysporum* and to reduce the dependence on the synthetic fungicides. These results of the present investigation are clear indication for potential of neem to control *Fusarium* spp. Joseph *et al.* (2008) showed neem in all concentration (5, 10 and 15%) has fungicide potential. Results showed significant suppression of both *M. incognita* and *F. oxysporum* by Neem seed kernel powder. Paul and Sharma (2002) reported that the leaf aqueous extract of neem inhibited the growth of soil born pathogenic fungi. NSKP was significantly effective against both the pathogens, and it may be due to presence of toxic chemicals in neem seed powder (Singh and Sitaramaiah, 1970).

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