Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

Journal of Entomology and Zoology Studies

E-ISSN: 2320-7078
P-ISSN: 2349-6800
www.entomoljournal.com
JEZS 2020; 8(2): 386-390

Received: 20-01-2020
Accepted: 24-02-2020

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Effect of oilcakes and bioagents on plant growth parameters on gladiolus infected by Root-knot nematode, *Meloidogyne incognita*

Tamireddy Anjali, Jayant Kumar Mahalik and Rudra Pratap Subudhi

Abstract
A pot culture experiment was conducted in the net house during *Rabi*, 2018-19 to find out suitable management practices for management of root-knot nematode, *Meloidogyne incognita* in Gladiolus. The treatments are T_1 (Bulb soaking with Carbosulfan 25 EC @ 0.2% for 6hrs), T_2 (soil application of *Purpureocillium lilacinum*@ 5kg/ha), T_3(Neem oil cake @ 2.0t/ha), T_4 (soil application of *Purpureocillium lilacinum*@ 2.5kg/ha+ Neem oil cake @ 1.0t/ha),T_5 (Carbofuran@ 1.0kga.i/ha), T_6(T_1+T_2), T_7(T_1+T_3), T_8(T_1+T_4), T_9(T_1+T_5), T_{10} (Untreated inoculated check). Each treatment was replicated thrice following Completely Randomized Design. The experimental result indicated that all the treatments significantly increased the plant growth parameters and decreased root-knot nematode population, number of egg masses as well as number of galls over untreated inoculated check. The combined applications gave better result than their single application. Among various treatments, T_8 (Bulb soaking with Carbosulfan 25 EC @0.2% for 6hrs followed by soil application of *Purpureocillium lilacinum* @2.5kg/ha along with Neem oil cake @ 1.0t/ha) resulted highest increase of plant height (95.2%), fresh shoot weight (84.3%), dry shoot weight (52.1%), root length (60.8%), fresh root weight (76.2%), dry root weight (78.2%) with corresponding decrease in number of root galls (82.1%), number of egg masses (89.6%) and root-knot nematode population (67.8%) over untreated inoculated check followed by T_9 (Bulb soaking with Carbosulfan 25 EC @0.2% for 6hrs + soil application of carbofuran @ 1.0kg a.i/ha). Therefore, Bulb soaking with Carbosulfan 25 EC @0.2% for 6hrs followed by soil application of *Purpureocillium lilacinum* @2.5kg/ha along with neem oil cake @ 1.0t/ha during planting was considered the most promising management option against root-knot nematode (*M. incognita*) affecting gladiolus crop.

Keywords: Gladiolus, Root-knot nematode, *Meloidogyne incognita*, *management*, oil cakes, neem cake

Introduction
Gladiolus (*Gladiolus grandiflorus*) belongs to family Iridaceae is an important perennial bulbous flowering plants grown commercially in many parts of the world for its fascinating flowers with variety of colours, huge form of florets and good keeping quality. It is popularly called as "Queen of bulbous flowers" [1]. As a cut flower it has earned its place of importance owing to its utility in bouquets, in indoor decoration and flower arrangements. India is elegantly paving a route to arise as a substantial participant in the global floriculture industry and is emerging as a future floral superpower. Gladioli are used as food plants by the larvae of some Lepidoptera species including the large yellow under wing, and gladiolus thrips.

Gladioli have been extensively hybridized and a wide range of ornamental flower colours are available from the many varieties. The main hybrid groups have been obtained by crossing between four or five species, followed by selection: 'Grandiflorus', 'Primulines' and 'Nanus'. They can make very good cut flowers for display.

Gladiolus is commercially grown in all state and presently gladiolus ranks only second to rose. At the domestic level so far as the state of Odisha is concerned, gladiolus ranks next to marigold with respect to acreage and at present it is grown over an area of 2245 ha with a production of 2182 lakh number of spikes. Commercial cultivation of this crop is being popularized by the department of Horticulture, Govt. of Odisha through National Horticulture Mission which has been implemented in the state since 2005.

The root-knot nematode is highly pathogenic to gladiolus crop and causes qualitative and quantitative decline in the spike production. Several factors lend to the severity of

Meloidogyne incognita in this crop because (a) Gladiolus is preferably grown on well drained sandy loam soil which favour survival and development of nematodes, (b) Root-knot nematodes invade the corms and cormels as well as the plant roots, thus persist from one season to the next in planting stocks and (c) Post-planting nematode management is complicated for applying chemicals with minimal disturbance of the root system of the plants and the horticultural practice of sweeping soil above the corm periodically during the growing season to control weeds [2]. So the pot culture experiment was undertaken to evaluate the effect of bioagent and oilcake alone or combination for management of root-knot nematode (*Meloidogyne incognita*) in gladiolus.

Materials and Methods

Experiments were conducted under pot culture condition in net house of Department of Nematology, College of Agriculture, O.U.A.T, Bhubaneswar during *Rabi* 2018-19. The entire experiment was done under pot culture condition under natural environmental condition. All the investigations and analysis were done in the P.G. laboratory of the department of Nematology of College of Agriculture. The experiment was laid out in 15cm diameter size earthen pots following Completely Randomized Design (CRD) with ten treatments, each replicated 3 times.

For culturing of nematodes, well pulverized sandy loam soil free from plant debris and gravels were collected from the University Central Farm. The soil was mixed thoroughly with sand and FYM in the ratio of 2:1:1 which was filled in gunny bags and autoclaved at 1.1kg/cm^2 pressure for one hour daily for two consecutive days. The sterilized soil was spread on a clean polythene sheet for 24 hours for renaturation of soil. In the meantime earthen pots of 15cm diameter were cleaned and surface sterilized in 1% formalin and made air dry. Gladiolus corms were surface sterilized in 2.5% Sodium hypochlorite solution for two minutes followed by rinsing corms thrice with distilled water and air dried in shade. The corms were sown in 15cm diameter earthen pots containing steam sterilized soil mixture. Three such pots were maintained in the net house. Light watering was done as and when necessary. Thinning was done at 3-4 leaf stage for keeping one plant per pot. A single egg mass of *Meloidogyne incognita* was collected from brinjal root and the population was multiplied on a susceptible brinjal grown in pots of 15 cm diameter containing sterilized soil.

Galled roots of brinjal plants were collected from the cultured pots. The roots were washed free from soil under a trap with gentle stream of water. After partial air drying egg masses were picked up with the help of tweezers and needles and kept over the wire gauge tissue paper assembly rested on the petridish containing clean tap water just touching the bottom surface of the wire gauge. The lid of petridish was covered to avoid evaporation loss. Freshly hatched second stage juveniles (J_2) were collected in beakers at 24 hour intervals and fresh water was added to the petridish at each change. Collection of juveniles was 26 continued for 7 to 8 days and used subsequently for experimental purpose. Water from the upper portion of the beaker was drained off without disturbing the nematodes at the bottom. These larvae were utilized to collection were stored in refrigerator at 8-10^o C for use within the next 7 to 8 days.

The nematode suspension containing second stage juveniles were collected in a beaker and taken out from refrigerator and left in the normal temperature for 1-2 hour for nematode reactive before counting. This was thoroughly stirred before taking a 2ml suspension in a rectangular counting dish to count the nematode, under a stereoscopic microscope (Southey, 1986) thrice. The average of total number of nematodes present in the 2ml stock sample was determined.

Infective second stage juvenile of root-knot nematode were inoculated @1000 J_2/kg soil. Three small holes of 2 cm depth were made with the help of glass rod in the soil closely around the root zone of the plant, into which measured volume of hatched juvenile suspension was slowly poured to release 1000 second stage juveniles per pot in all the treatments followed by closing the hole immediately. Then a light watering was done.

Different treatments and methodology were detailed below for the experiment. Experiment was conducted to study the efficacy of some commonly used oil cakes i.e. neem oil cakes against *Meloidogyne incognita* infecting gladiolus. A standard check with neem oil cake was thoroughly mixed in the pot followed by a light watering. An inoculated check was also maintained. Hence, the experiment was laid in a Completely Randomized Design with ten treatments, each replicated thrice. The treatments were as follows:

T_1: Bulb soaking with Carbosulfan 25 EC @0.2% for 6hrs.
T_2: Soil application of *Purpureocillium lilacinum* @5kg/ha
T_3: Neem oil cake @2.0t/ha
T_4: Soil application of *Purpureocillium lilacinum* @2.5kg/ha+Neem oil cake @1.0t/ha
T_5: Carbofuran 3G @ 1.0 kg a.i/ha
T_6: T_1+T_2
T_7: T_1 +T_3
T_8: T_1+T_4
T_9: T_1+T_5
T_{10}: Untreated inoculated check

Each oil cake were applied as per required doses and mixed thoroughly to the autoclaved soil (1kg) filled with surface sterilized 15cm diameter earthen pots followed by light watering. All these pots were maintained as such in the net house for the period of 15 days for proper decomposition of oil cakes.

Gladiolus corms (cv. Candyman) raised in sterilized soil was transplanted in the pots (one in each). Then 7days after transplanting thousand freshly hatched second stage juvenile of *Meloidogyne incognita* were inoculated to each pot followed by light watering. This experiment was terminated after 45 days of nematode inoculation during which care of young seedlings and other intercultural operations were attended regularly.

Recording of observation

At 45 days after inoculation of *M. incognita*, each plant was removed from the post soil carefully. Roots were washed free from soil and other adhering particles under slow stream of water and observation were recorded on different plant growth parameters, number of galls and nematode population in soil as well as in root. shoot length, root length, spike length, fresh shoot weight, dry shoot weight, root length, fresh root weight, dry weight of root and number of galls were identified. Estimation of nematode from soil was done by cob's sieving technique (Cobb's, 1918) and modified Baermann funnel technique (Schindler, 1961). estimation of nematode population in root is done by Byrd method (Byrd *et al.*, 1983).

Statistical analysis

Various observation recorded during the course of investigation were subjected to statistical analysis in a Completely Randomized Design. Data on number of root galls, egg masses and final nematode population in soil as well as in root were analyzed after square root and log transformation respectively. Fisher's methods of analysis of variance at 5% level of significance were followed. The difference between the two treatments means if greater than the LSD value indicated the significant difference between the treatments. In this way comparison between the treatments was made.

Results and Discussion

From Table-1, it was evident that all treatments of exhibited significant increase in plant growth parameters (shoot & root length, spike length, fresh & dry shoot weight, fresh & dry root weight) Shoot length was maximum in T_8 (Bulb soaking with Carbosulfan 25 EC @0.2% for 6hrs and soil application of *Purpureocillium lilacinum* @2.5kg/ha+Neem oil cake @1.0t/ha) with increase with the tune of 39.54% over T_{10}. This was followed in descending order as T_9 (34.81%), T_6 (32.17%), T_7 (30.17%), T_4 (26.56%), T_5 (25.09%), T_2 (22.85%), T_1 (19.82%), T_3 (18.11%) over check. Among different treatments, maximum plant height (95.27cm) was recorded in T_8 which was statistically different from rest treatments followed by T_9 (92.03 cm), T_6 (90.23cm), T_7 (88.87 cm), T_4 (86.40 cm), T_5 (85.40cm), T_2 (83.87cm), T_1 (81.80cm) and T_3 (80.63cm) in descending order. The treatment T_8 exhibited highest spike length (62.83cm) was statistically different from other treatments T_9 (59.23cm), T_6 (57.43cm), T_7 (55.33cm), T_4 (53.40cm), T_5 (52.53cm), T_2 (50.73cm), T_1 (48.37cm) and T_3 (46.97cm) in descending order. The highest increase (47.60%) over check (42.57cm) was observed in T_8, which was closely followed by T_9, T_6, T_7, T_4, T_5, T_2, T_1 and T_3. The treatment T_8 exhibited highest fresh shoot weight (84.33g) was statistically different from other treatments T_9 (82.40g), T_6 (79.50g), T_7 (77.93g), T_4 (76.47g), T_5 (74.23g), T_2 (72.53g), T_1 (70.00g) and T_3 (67.63g) in descending order. The highest increase (31.16%) over check (64.30g) was observed in T_8, which was closely followed by T_9, T_6, T_7, T_4, T_5, T_2, T_1 and T_3. The maximum increase in dry shoot weight over check was noticed in T_8 (52.15%) which was closely followed by T_9 (46.50%), T_6 (38.01%), T_7 (32.95%), T_4 (27.83%), T_5 (21.76%), T_2 (16.35%), T_1(12.59%) and T_3 (6.90%). However, highest dry shoot weight (10.51g) was recorded in the plant treated with Bulb soaking with Carbosulfan 25 EC @0.2% for 6hrs and soil application of *Purpureocillium lilacinum* @2.5kg/ha+Neem oil cake @1.0t/ha (T_8) followed by T_9 (10.12g), T_6 (9.54g), T_7 (9.19),T_4 (8.83 g), T_5(8.41g), T_2(8.04 g) and T_1(7.78g) and T_3(7.39g) in descending order.

Table 1: Effect of Carbosulfan, *Paecilomyces lilacinum* and Neem oil cake on the shoot growth of *Gladiolus spp.* infected by *M. incognita.*

Treatment	Plant height (cm)	Increase over check (%)	Spike length (cm)	Increase over check (%)	Fresh shoot weight (g)	Increase over check (%)	Dry shoot weight (g)	Increase over check (%)
T_1	81.80	19.82	48.37	13.62	70.00	8.86	7.78	12.59
T_2	83.87	22.85	50.73	19.18	72.53	12.80	8.04	16.35
T_3	80.63	18.11	46.97	10.33	67.63	5.18	7.39	6.90
T_4	86.40	26.56	53.40	25.44	76.47	18.92	8.83	27.83
T_5	85.40	25.09	52.53	23.40	74.23	15.45	8.41	21.76
T_6	90.23	32.17	57.43	34.92	79.50	23.64	9.54	38.01
T_7	88.87	30.17	55.33	29.98	77.93	21.20	9.19	32.95
T_8	95.27	39.54	62.83	47.60	84.33	31.16	10.51	52.15
T_9	92.03	34.81	59.23	39.14	82.40	28.15	10.12	46.50
T_{10}	68.27	-	42.57	-	64.30	-	6.91	-
SE (m) ±	0.86	-	0.71	-	0.57	-	0.13	-
CD(0.05)	2.55	-	2.10	-	1.67	-	0.37	-

From Table-2, T_8 registered highest root length (42.93cm) which was found statistically different from the rest of the treatments followed by T_9 (40.30cm) T_6 (39.33cm), T_7 (38.23cm), T_4 (35.20cm), T_5 (34.53cm) and T_2 (34.00cm) T_1 (32.83 cm) T_3 (30.80cm) in descending order. Percentage increase of mean root length over check (26.70cm) was observed highest in T_8 (60.80%), followed by T_9 (50.94%), T_6 (47.32%), T_7 (43.20%), T_4 (31.84%), T_5 (29.34%), T_2 (27.34%), T_1 (22.97%) and T_3 (15.36%).The fresh root weight in T_8 was highest (19.13g) followed by T_9 (18.33g), T_6 (17.75g), T_7 (16.88g), T_4 (15.29g), T_5 (14.47g), T_2 (13.01g) T_1 (12.04 g) and T_3 (11.80g) in descending order. The maximum being recorded in T_8 followed by T_9 (68.97%), T_6 (63.56%), T_7 (55.55%), T_4 (40.89%), T_5 (33.33%), T_2 (19.88%), T_1 (11.00%) and T_3 (8.79%) in descending order. T_8 registered highest dry root weight (3.24g) and found statistically different from the rest of the treatments followed by T_9 (3.06g), T_6 (2.85g), T_4 (2.35g), T_5 (2.30g), T_2 (2.19g), T_1 (2.14g) and T_3 (2.05g) in descending order.

Table 2: Effect of Carbosulfan, *Paecilomyces lilacinum* and Neem oil cake on the root growth of *Gladiolus spp* infected by *M. incognita.*

Treatments	Root length (cm)	Increase over check (%)	Fresh root weight (g)	Increase over check (%)	Dry root weight (g)	Increase over check (%)
T_1	32.83	22.97	12.04	11.00	2.14	17.77
T_2	34.00	27.34	13.01	19.88	2.19	20.33
T_3	30.80	15.36	11.80	8.79	2.05	12.64
T_4	35.20	31.84	15.29	40.89	2.35	28.94
T_5	34.53	29.34	14.47	33.33	2.30	26.19
T_6	39.33	47.32	17.75	63.56	2.85	56.78
T_7	38.23	43.20	16.88	55.55	2.59	42.31
T_8	42.93	60.80	19.13	76.28	3.24	78.21

T_9	40.30	50.94	18.33	68.97	3.06	68.13
T_{10}	26.70	-	10.85	-	1.82	-
SE(m)±	0.68	-	0.28	-	0.06	-
CD(0.05)	2.00	-	0.82	-	0.17	-

From Table-3, The treatment T_8 (Carbosulfan 25 EC @0.2% for 6hrs and Application of *Purpureocillium lilacinum* @ 2.5kg/ha+ Neem oil cake @ 1.0t/ha) recorded the lowest number of galls (22.00) and found significantly different from rest of the treatments followed by T_9 (28.33), T_6 (32.67), T_7 (34.33), T_4 (44.67), T_5 (48.33), T_2 (64.67), T_1 (82.33) and T_3 (90.67) respectively in increasing order. The highest reduction galls per plant was observed in T_8 (82.11%) followed by T_9 (76.96%), T_6 (73.44%), T_7 (72.09%), T_4 (63.69%), T_5 (60.70%), T_2 (47.43%) T_1(33.06%) and T_3(26.29%) in descending order. The highest reduction of *M. incognita* population (67.80%) over untreated check recorded in T_8 followed by T_9 (61.34%), T_6 (55.44%), T_7 (51.59%), T_4 (45.80%), T_5 (43.20%), T_2 (41.95%), T_1 (34.47%) and T_3 (32.48%) in descending order. The highest (89.67%) reduction in egg mass over check (109.67) was observed in T_8 which was significantly different from other treatments followed by T_9 (84.20%), T_6 (80.24%), T_7 (78.72%), T_4 (59.27%), T_5 (55.93%), T_2 (55.93%), T_1 (38.30%) and T_3 (30.70%) over untreated check (T_{10}) in decreasing order. The egg mass produced by *M. incognita* was lowest (11.33) in T_8 followed by T_9 (17.33), T_7 (23.33), T_6 (21.67), T_4 (44.67), T_5 (48.33), T_2 (48.33), T_1 (67.67) and T_3 (76.00) in increasing order. Root-knot nematode population per 200 cc soil was highest in untreated check (1176.00) and lowest in (378.67) in T_8 followed by T_9 (454.67), T_6 (524.00), T_7 (569.33), T_4 (637.33), T_5 (668.00) T_2 (682.67) T_1 (770.67) and T_3 (794.00) in increasing order.

Table 3: Effect of Carbosulfan, *Paecilomyces lilacinum* and Neem oil cake on the *M. incognita* growth in *Gladiolus.*

Treatment	Galls/ Plant	Decrease over check (%)	Egg masses/ plant	Decrease over check (%)	Nematode population (J_2/200cc soil)	Decrease over check (%)
T_1	82.33	33.06	67.67	38.30	770.67(27.76)*	34.47
T_2	64.67	47.43	48.33	55.93	682.67(26.13)	41.95
T_3	90.67	26.29	76.00	30.70	794.00(28.18)	32.48
T_4	44.67	63.69	44.67	59.27	637.33(25.24)	45.80
T_5	48.33	60.70	48.33	55.93	668.00(25.84)	43.20
T_6	32.67	73.44	21.67	80.24	524.00(22.89)	55.44
T_7	34.33	72.09	23.33	78.72	569.33(23.86)	51.59
T_8	22.00	82.11	11.33	89.67	378.67(19.45)	67.80
T_9	28.33	76.96	17.33	84.20	454.67(21.31)	61.34
T_{10}	123.00	-	109.67	-	1176.00(34.28)	-
SE(m) ±	1.86	-	1.55	-	0.34	-
CD(0.05)	5.48	-	4.57	-	1.01	-

The results obtained on reduction of nematode population by neem cake and pongamia cakes were in agreement with those of Mishra *et al.* (1989) [3] who reported that aqueous extracts of neem and mustard cake against *M. incognita* reduced the hatching ability of eggs. Bhattacharya and Goswami (1989) [3] who observed that significant increase in plant growth and reduction in root galling by *M. incognita* with neem cake at 4 per cent concentration. Further, neem cake significantly increased plant growth of tomato and reduced nematode infestation.

Nagesh *et al.* (2001) [4] reported that maximum parasitization of eggs and egg masses of *M. incognita* were by *P. lilacinus*. The reason for reduction in galls and egg masses might be due to parasitic activity of *Paecilomyces lilacinus* on eggs and all stages of nematodes. The developing *P. lilacinus* kills the nematode by feeding on its body contents. In effect, the *P. lilacinus* acts as a parasite on the all stages of nematode. The above results with respect to bio-agents in increasing floral characteristics and flower yield are in conformity with the findings of Rao *et al.* (2003) [8], Rao *et al.* (2004) [7] who observed increased number of florets/spike, spike /plot and floral characteristics in bio-agent treated plots.

The increased shoot growth, yield and other parameters observed here could be attributed to the release of growth promoting substances by bio-agents (Baker *et al.*, 1986) [2] or by producing toxic metabolites which inhibit nematodes and exclude other deleterious microorganisms. In a different experiment, Ashraf and Khan (2010) [1] stated that the efficacy of the biocontrol agent *Paecilomyces lilacinus* against *M. javanica* attacking eggplant increased in the presence of oil cake.

Fig 1: Effect of Carbosulfan, *Paecilomyces lilacinum* and Neem oil cake on the shoot growth of *Gladiolus* infected by *M. incognita*

Fig 2: Effect of Carbosulfan, *Paecilomyces lilacinum* and Neem oil cake on the root growth of *Gladiolus* infected by *M. incognita.*

Conclusion

From the above result, it was concluded that the treatment T_8 (Bulb soaking with Carbosulfan 25 EC @ 0.2% for 6 hours and soil application of *Purpureocillium lilacinum*@ 2.5 kg/ha + Neem oil cake @ 1.0t/ha) was effective in increasing plant growth parameters like shoot length, spike length, fresh and dry weights of shoots and roots as well as decreasing the number of galls, larval population in soil. This management module can be recommended to the farmers growing gladiolus for management of root-knot nematode, *M. incognita*.

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