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Effect of AM fungus and oilcakes with different doses of fertilizers on root knot nematode (*Meloidogyne incognita*) infecting black gram

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Abstract
A replicated pot culture experiment was conducted for studying the effect of AM fungus and oilcakes with different doses of fertilizers on root knot nematode (*Meloidogyne incognita*) infecting black gram. All treatments significantly increased the plant growth parameters, uptake of phosphorus by plant, growth of AM fungus, extent of colonization of AM fungus in black gram roots and reduced root knot nematode population as well as number of galls over check. However, application of mustard cake @ 100g/m^2 + AM fungus @ 5g/m^2 along with 50% P and RDNK results more increase in growth parameters of both shoot and root, uptake of phosphorous by plants, colonization of AM fungus in roots and growth of AM fungus with corresponding decrease in number of root galls by and root knot nematode population over check. Thus application of mustard cake @ 100g/m^2 and AM fungus @ 5g/m^2 along with 50% P and RDNK could be the most promising management option against root knot nematode (*Meloidogyne incognita*) infecting black gram.

Keywords: oilcake, mustard cake, AM Fungus, *Meloidogyne incognita*, black gram

1. Introduction
Black gram (*Vigna mungo* L.) is an important short duration pulse crop grown mainly in tropical and subtropical countries. It is supposed to have originated in India, as evident from Vedic literature. Apart from the sole crop, it is also grown as a mixed crop, catch crop, sequential crop under residual moisture condition after harvesting of rice and also before and after the harvest of summer crops under semi irrigated and dryland conditions. It is a 90-120 days duration crop.

Among the major biotic factors that affect the black gram production, root knot nematode (*Meloidogyne incognita*) is the most serious pest causing considerably low yield in all parts of India. It ranks top position among various plant parasitic nematodes infecting black gram because of its wide adaptation in tropical and temperate areas.

Root knot nematode causes 17 to almost 23% loss in yield of black gram [1]. Among various management practices against root knot nematode, the ecofriendly and economically viable measure is the cultural method of management, which can be adopted by common farmers. In this context use of oilcakes with the application of AM fungus is gaining importance. Oilcakes change the physical and chemical properties of the soil which makes the soil atmosphere unfavorable for nematode activity and improves soil condition for greater root growth there by increasing the utilization of soil nutrients. There is a report on the combined application of neem oilcakes and *Glomus fasciculatum* for management of *Meloidogyne incognita* in brinjal [2].

2. Materials and Methods
A pot culture experiment was laid out under net house condition in Department of Nematology, College of Agriculture, OUAT, Bhubaneswar during *kharif*, 2015 in 15 cm size earthen pots following Complete Randomized Design (CRD) with 10 treatments, each replicated thrice.

The treatments were as follows: T_1 – AM Fungus (*Glomus fasciculatum*) @ 5g/m² with 50% P & RDNK, T_2 – AM Fungus (*Glomus fasciculatum*) @ 5g/m² with 100% RDF, T_3 – Neem cake (*Azadirachta indica*) @ 100g/m² with 100% RDF, T_4 – Mustard cake (*Brassica campestris*) @ 100g/m² with 100% RDF, T_5 - T_1 + Neem cake @ 100g/m^2, T_6 – T_1 + Mustard cake @ 100g/m^2, T_7 – T_2 + Neem cake @ 100g/m^2, T_8 – T_2 + Mustard cake @ 100g/m^2,

T_9 – Carbofuran as standard check @ 1 kg a.i/ha with 100% RDF, T_{10} – Untreated inoculated check with 100% RDF.

Pots were filled with 1 kg autoclaved sterilized soil + sand + FYM mixture in 2:1:1 ratio each. Oilcakes were first mixed with sterilized soil in replicated pots of the respective treatments i.e. Neem oilcake (T_3, T_5, T_7) and Mustard oilcake (T_4, T_6, T_8) at least 3 weeks prior to sowing of black gram seeds in pots. Black gram cv *"Mahuri"* seeds surface sterilized in 2.5% sodium hypochlorite solution for two minutes followed by rinsing seeds thrice with distilled water and air dried in shade were sown in pots. At the time of sowing seeds, AM fungus was inoculated in the relevant pots after removing top 2cm soil. Five grams AM fungus culture (*Glomus fasciculatum*) in sand @600 chlamydospores/pot was spread over the soil in replicated pots of the respective treatments (T_1, T_2, T_5, T_6, T_7 & T_8) and covered with sterilized soil. Light watering was done to keep the soil moist. Seeds when germinated and attained 3-4 leaf stage, thinning was done keeping one plant per pot. Then the application of *Meloidogyne incognita* was done @ 1000 J_2/ kg of soil in all treatments.

Data on plant growth parameters, soil and plant nematode population, uptake of phosphorus by plant and AM fungus infection were recorded at 60 days after sowing seed. Tabular data were subjected to statistical analysis for each parameter for comparison of different treatments following Fisher's method of analysis of variance at 5% level.

3. Results and Discussion

The effect of AM fungus and oilcakes with different doses of fertilizers was estimated on the basis of the differential changes in plant growth parameters (Plant height, fresh & dry shoot weight, Root length, and fresh & dry root weight), uptake of phosphorus by plant, AM fungus infection and nematode infection parameter as number of galls, nematode population in soil & root.

The data (Table 1) indicated that there was progressive increase in plant growth parameters in respect of shoot length, fresh and dry shoot weight, root length, fresh and dry root weight and phosphorus uptake by plant in all treatments over untreated check. There was highest record on increase in mean shoot length (32.2cm) in the treatment (T_6) having combination of mustard cake @ 100g/m^2 + AM fungus @ 5g/m^2 along with 50% P and RDNK with an increase to the tune of 45.04% over untreated check (22.2cm), followed by (T_8) having combination of mustard cake @ 100g/m^2 + AM fungus 5g/m^2 with 100% RDF (30.8cm), T_5 (30.3cm). Mean root length in all treated black gram plants increased over check. T_6 (mustard cake @ 100g/m^2 + AM fungus @ 5g/m^2 along with 50% P and RDNK) registered highest root length (27.66cm) followed by T_8 (27.23cm), T_5 (25.66cm) and T_4 (25.16cm). Percentage of increase in root length was found highest in (T_6) with combination.

Table 1: Influence of *M. incognita*, Oilcakes and AM fungus either alone or in combination on growth parameters and Phosphorus uptake by plant (mg/plant) of Black gram cv. Mahuri

Treatment	Shoot Length (cm)	% increase over check	Fresh Shoot Weight (g)	% increase over check	Dry Shoot Weight (g)	% increase over check	Root length (cm)	% increase over check	Fresh Root Weight (g)	% increase over check	Dry Root Weight (g)	% increase over check	'P' uptake by plant (mg/plant)	% increase over check
T_1 (AM Fungus @ 5g/m² with 50% P & RDNK)	22.8	2.70	4.96	7.82	0.56	5.66	19.5	2.63	0.9	8.43	0.25	8.69	1.0	25
T_2 (AM Fungus @ 5g/m² with 100% RDF	23.2	4.50	5.10	10.86	0.63	18.86	20.33	7.00	0.96	15.66	0.26	13.04	1.1	37.5
T_3(Neem cake @ 100g/m² with 100% RDF)	26.8	20.72	6.26	36.08	0.70	32.07	23.16	21.89	1.1	32.53	0.30	30.43	0.8	0
T_4(Mustard cake @ 100g/m² with 100% RDF)	29.2	31.53	6.80	47.82	0.83	56.60	25.16	32.42	1.3	56.62	0.33	43.47	0.8	0
T_5(T1+Neem cake @ 100g/m^2)	30.3	36.48	7.3	58.69	0.96	81.13	25.66	35.05	1.43	72.28	0.36	56.52	1.4	75
T_6(T1+Mustard cake @ 100g/m^2)	32.2	45.04	8.2	78.26	1	88.67	27.66	45.57	1.7	104.81	0.43	86.95	1.5	87.5
T_7(T2+Neem cake @ 100g/m^2)	27.8	25.22	6.46	40.43	0.80	50.94	23.5	23.68	1.13	36.14	0.33	43.47	1.2	50
T_8(T2+Mustard cake @ 100g/m^2)	30.8	38.73	7.60	65.21	0.96	81.13	27.23	43.31	1.5	80.72	0.40	73.91	1.3	62.5
T_9(Carbofuran@1kg a.i/ha with 100% RDF)	24.1	8.55	5.66	23.04	0.66	24.52	21.33	12.26	1.03	24.09	0.30	30.43	0.8	0
T_{10}(Untreated check with 100% RDF)	22.2		4.60		0.53		19		0.83		0.23		0.8	
SE(m)±	2.39		0.64		0.12		2.14		0.24		0.07		0.08	
L.S.D (0.05)	5.19		1.41		0.27		4.65		0.54		0.17		0.19	

Of mustard cake @ 100g/m^2 + AM fungus @ 5g/m^2 along with 50% P and RDNK (45.57%) followed by T_8 (43.31%), T_5 (35.05%), T_4 (32.42%), the lowest being recorded in T_1 (2.63%). Mustard cake @ 100g/m^2 + AM fungus @ 5g/m^2 along with 50% P and RDNK (T_6) exhibited highest mean fresh shoot weight (8.2g) with 78.26% increase over check (T_{10}) which was closely followed by (T_8) mustard cake @ 100g/m^2 + AM fungus 5g/m^2 with 100% RDF (65.21%) and (T_5) AM Fungus @ 5g/m² with 50% P & RDNK +Neem cake @ 100g/m^2 (58.69%), the lowest being 7.82% increase in T_1. While observing the data on fresh root weight, it was noticed that mustard cake @ 100g/m^2 + AM fungus @ 5g/m^2 along with 50% P and RDNK (T_6) recorded the highest mean fresh root weight (1.7g) followed by T_8 (1.5g), T_5 (1.43g) and T_4 (1.3g), the lowest being in T_{10} (0.83g). Percentage of increase in fresh root weight was recorded highest in T_6 (104.81%) followed by T_8 (80.72%), T_5 (72.28%), T_4 (56.62%), the lowest being in T_1 (8.43%). The percentage of increase in dry

shoot weight over check ranged from 5.66% (T_1) to 88.67% (T_6). Mean dry shoot weight in T_6 (1.00g) was found statistically non-significant withT_4 (0.83g), T_5 (0.96g), T_7 (0.80g) and T_8 (0.96g). However, combined application of AM fungus @ 5g/m^2 with 50% P and RDNK along with mustard cake @ 100g/m^2 in T_6 indicated highest percentage of increase in dry shoot weight (88.67%) followed by T_5 & T_8 (81.13%) and T_4 (56.6%) over check (T_{10}). Dry mean root weight in different treatments ranged from 0.23g in T_{10} to highest 0.43g in T_6. However, there was highest percentage of increase in dry root weight in T_6 (86.95%), followed by T_8 (73.91%), T_5 (56.52%), T_4 and T_7 (43.47%) over check. Uptake of available phosphorus by plant during termination of the experiment indicated that combined application of AM fungus @ 5g/m^2 with 50% P and RDNK along with mustard cake @ 100g/m^2 in T_6 exhibited highest uptake of phosphorus (1.5mg) followed by T_5 (1.4mg), T_8 (1.3mg), the lowest uptake in T_3, T_4, T_9 and T_{10} (0.8mg). Percentage of increase in uptake of phosphorus content by black gram plant was found maximum in T_6 (87.5%) followed by T_5 (75%), T_8 (62.5%) & T_7 (50%) over check. This results in agreement with earlier reports of [3, 4] who proved the effectiveness of oilcakes improving plant growth in different crops due to stimulation of microbial activity in the rhizosphere during decomposition of oilcakes and facilitating better availability of organic source of macro and micro nutrients to black gram plants promoting better growth of plant. Rapid multiplication of AM fungus in mustard cake amended soil supplemented with fertilizers providing better supply of nutrients to boost the plant growth and yield. This is in agreement with [5] who indicated that *Glomus fasciculatum* increased the nitrogen and phosphorus content of brinjal and [6] reported that influence of *G. fasciculatum* increased phosphorus content of root and shoot of blackgram while studying interaction of *G. fasciculatum* & *M. incognita* on blackgram. AM fungus playing a vital role as plant growth promoters providing better nutrition in shape of increased availability of phosphorus, nitrogen, potassium, calcium, copper, magnesium, manganese and zinc which might have supplemented growth and spread more new roots helping more uptake of nutrients & water supply to plant [7, 8].

So far as population growth of AM fungus is concerned, the data (Table 2) indicated that there was maximum multiplication of chlamydospores in T_6 (8268) followed by T_8 (6378) and T_5 (4012), the lowest being recorded in T_1 (2219). T_6 where AM fungus @ 5g/m^2 with 50% P & RDNK and mustard cake @ 100g/m^2 were applied there was highest reproductive growth (13.78) of chlamydospores followed by T_8 (10.63), T_5 (6.68) and T_7 (6.32). But T_1 & T_2 where AM fungus @ 5g/m^2 alone with different doses of fertilizers was inoculated, the growth factor of chlamydospores were relatively less i.e. 3.69 and 5.2 respectively. Thus it was noticed that combination of AM fungus with oilcakes supported considerable multiplication of chlamydospores as compared to AM fungus inoculated alone. Mean data in various treatments indicated that T_6, where AM fungus @ 5g/m^2 with 50% P & RDNK along with mustard cake @ 100g/m^2 were applied, exhibited highest root colonization (73.33%) of AM fungus followed by

Table 2: Population Growth of AM Fungus (*Glomus fasciculatum*) in Black gram cv. Mahuri

Treatment	Initial AM Fungus Population	Final AM Fungus Population	RF	AM Fungus colonization in Root (%)
T_1 (AM Fungus @ 5g/m² with 50% P & RDNK)	600	2219 (3.34)*	3.69	40 (39.50)**
T_2 (AM Fungus @ 5g/m² with 100% RDF	600	3120 (3.49)	5.2	50 (45.29)
T_3(Neem cake @ 100g/m² with 100% RDF)	0	0	-	0
T_4(Mustard cake @ 100g/m² with 100% RDF)	0	0	-	0
T_5(T1+Neem cake @ 100g/m^2)	600	4012 (3.60)	6.68	60 (51.08)
T_6(T1+Mustard cake @ 100g/m^2)	600	8268 (3.91)	13.78	73.33 (59.38)
T_7(T2+Neem cake @ 100g/m^2)	600	3792 (3.55)	6.32	55 (48.37)
T_8(T2+Mustard cake @ 100g/m^2)	600	6378 (3.79)	10.63	70 (57.15)
T_9(Carbofuran@1kg a.i/ha with 100% RDF)	0	0	-	0
T_{10}(Untreated check with 100% RDF)	0	0	-	0
SE(m)±		0.05		2.07
L.S.D (0.05)		0.13		4.50

*Figures in parentheses are log transformed values

** Figures in parentheses indicated transformed angular values

T_8(70%), T_5(60%) and T_7(55%). As compared to AM fungus treated alone in T_1 & T_2, there was progressive rise in root colonization of AM fungus in T_7, T_5, T_8 & T_6 where there was combined application of AM fungus with oilcakes. This evidence collaborates with findings of [9] who reported that *G. fasciculatum* inoculated 10 days prior to *M. incognita* in blackgram exhibited increased percentage of root colonization of AM fungus compared to other treatments.

Table 3 revealed that there was highest percentage of reduction in number of galls in T_6 (88.53%), T_7 (70.56%) and T_3 (62.12%) over check in descending order, the lowest being 35.93% in T_1 over check. Also it was noticed that treatments where AM fungus were treated alone with different doses of fertilizers, the number of galls produced by root knot nematode were comparatively more than the corresponding treatments where AM fungus and oilcakes applied together with different doses of fertilizers and also where oilcakes with RDNPK were applied. As per the data on mean nematode population in soil and root, it was evident that T_6 exhibited the lowest root knot nematode population (342) with highest reduction (83.27%) over check followed by T_8 (79.16%), T_5 (78.04%), T_4 (76.28%) & T_7 (74.37%) in descending order over check. As compared to check (T_{10}), all other treatments significantly contributed in reducing the root knot nematode population. As regards to growth factor, mustard cake @100g/m^2 + AM fungus @ 5g/m^2 along with 50% P and RDNK (T_6) registered the least growth of root knot nematode population (0.34) followed by T_8 (0.43), T_5 (0.45), T_4 (0.49) and T_7 (0.52) in ascending order.

Table 3: Root Knot Nematode (*M. incognita*) infection parameters and its population growth in Black gram cv. Mahuri

Treatment	No. of Galls	% decrease over check	RKN population (Soil and Root)	% decrease over check	RF
T_1 (AM Fungus @ 5g/m² with 50% P & RDNK)	98.66 (9.91)*	35.93	578 (2.75)**	71.73	0.58
T_2 (AM Fungus @ 5g/m² with 100% RDF	73.66 (8.58)	52.16	693 (2.83)	66.11	0.69
T_3(Neem cake @ 100g/m² with 100% RDF)	58.33 (7.61)	62.12	835 (2.91)	59.16	0.84
T_4(Mustard cake @ 100g/m² with 100% RDF)	37.66 (6.12)	75.54	485 (2.66)	76.28	0.49
T_5(T1+Neem cake @ 100g/m^2)	26.00 (5.09)	83.11	449 (2.64)	78.04	0.45
T_6(T1+Mustard cake @ 100g/m^2)	17.66 (4.19)	88.53	342 (2.50)	83.27	0.34
T_7(T2+Neem cake @ 100g/m^2)	45.33 (6.72)	70.56	524 (2.69)	74.37	0.52
T_8(T2+Mustard cake @ 100g/m^2)	24.33 (4.92)	84.20	426 (2.62)	79.16	0.43
T_9(Carbofuran@1kg a.i/ha with 100% RDF)	58.66 (7.65)	61.90	724 (2.85)	64.59	0.72
T_{10}(Untreated check with 100% RDF)	154.00 (12.38)		2045 (3.30)		2.05
SE(m)±	0.45		0.09		
L.S.D (0.05)	0.98		0.20		

*Figures in parentheses are log transformed values

** Figures in parentheses indicated transformed angular values

Highest growth of root knot nematode population was observed in T_{10} (2.05). During decomposition process of oilcakes, nematoxic compounds released in soil might be nematostatic or nematicidal to root knot nematode reducing its population and other infection parameters. This is in agreement with earlier reports of [10] who proved the effectiveness of oilcakes improving plant growth and reducing nematode population. Moreover, the reduction in root knot nematode population might be due to spread of more mycelia net of AM fungus inside root cortex of black gram root creating physical hindrance, curbing entry and infection by root knot nematode as suggested by [11]. Moreover, reduction of root knot nematode population may be due to accumulation of nematotoxic substances particularly allyl isothiocyanate by mustard decomposition as suggested by [12] and [13]. Also [14] indicated preoccupation of host root by mycorrhizal hyphae coupled with biochemical changes in the mycorrhizal affected root system increased lignin and phenol production making the tomato plant resistant to *Meloidogyne javanica*.

4. Conclusion

From the experimental findings it was evident that all treatments increased the plant growth parameters, yield attributes, enhanced uptake of phosphorous by plant and reduced root knot nematode population as well as galls over check. Application of mustard cake @ 100g/m^2 + AM fungus @5g/m^2 along with 50% P and RDNK in T_6 is considered the most promising management option against root knot nematode (*Meloidogyne incognita*) infecting black gram.

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