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# INFLUENCE OF OIL CAKES IN COMBINATION WITH INORGANIC FERTILIZERS ON GROWTH AND SPORULATION OF *VERTICILLIUM LECANII*

by
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**Summary**. The supplementation of oil cakes with nitrogen, phosphorus and potassium in the form of inorganic fertilizers, had a synergistic effect on the growth, behaviour and sporulation of *Verticillium lecanii*. Of three oil cakes, neem was the best substrate for *V. lecanii* in terms of amount and duration of mycelial growth followed by karanj. The supplementation of the oil cakes with N, P and K enhanced the mycelial mat weight, duration of mycelial growth and sporulation of *V. lecanii*.

The application of inorganic fertilizers and oil cakes is normal practice in crop production, but their effect on the behaviour of any antagonist has not been studied. Therefore, *in vitro* studies were conducted to evaluate the role of organic amendments (oil cakes) in combination with inorganic fertilizers on the growth and sporulation of the antagonistic fungus, *Verticillium lecanii* Zimm.

## Materials and methods

One gram each of finely powdered and sieved oil-cakes of castor (*Ricinus communis* L.), karanj (*Pongamia pinnata* Merr.) and neem (*Azadirachta indica* A. Juss.) were placed in 50 ml conical flasks containing 10 ml distilled water to make 10% oil cake suspensions. The following fertilizers were added to the suspensions (w/v of 10% oil cake suspensions), alone or in combinations: nitrogen (N), 1% (in the form of ammonium nitrate); phosphorus (P), 0.5% (single super phosphate); potassium (K), 0.5% (murate of potash); N (1%) + P (0.5%); N (1%) + K (0.5%); P (0.5%) + K (0.5%) and N+P+K (1:0.5:0.5). All of the flasks were then autoclaved. One loopful of *Verticillium lecanii* pure culture (SBI isolate) was then inoculated in each conical flask under aseptic conditions; and the flasks were then incubated at 22 °C. The growth of *V. lecanii* in each flask (i.e., area of mycelial mat cover/area of suspension in the flask) was recorded at weekly intervals for seven weeks after inoculation and rated on a 0-100 scale. The mycelial mat from each flask was collected and weighed. Another set of *V. lecanii* inoculated flasks with same treatments were incubated for eight weeks and the spore count was recorded in each treatment with a haemocytometer after serial dilution. Each treatment was replicated five times.

## Results and discussion

The mycelial growth recorded over a period of seven weeks (Table I) differed between the

three oil cakes. The mycelial growth was 40% on castor and neem cake suspensions, but on karanj cake suspension it had not initiated by the end of the first week. The mycelial growth of *V. lecanii* on castor cake suspension remained at 40% from the first to fifth week and it then disappeared by the sixth week. The mycelial growth was maximum after four weeks on karanj and neem cake suspensions (65 and 80%, respectively) and declined from the fifth week. These observations indicate that among the three oil cakes, neem served as a better substrate for *V. lecanii* mycelial growth in terms of amount (maximum of 80% at the fourth week) and duration (for seven week of incubation) followed by karanj cake.

The mycelial growth pattern of *V. lecanii* on oil cake substrates supplemented with K, N+P, N+K and P+K was similar to that on oil cakes without any nutrient supplementation. However, the growth pattern on oil cakes with P and N+P+K was significantly different from that on the oil cake and nutrient combinations discussed above. The addition of P to the oil cake suspensions significantly increased the amount and duraion of mycelial growth of *V. lecanii* (Table I). Phosphorus supplementation doubled *V. lecanii* growth on castor cake suspension (three and four weeks of incubation) and increased it to 80% on karanj cake suspension (four to six weeks of incubation) and to 100% on neem cake suspension (four and five weeks of incubation).

When castor, karanj and neem cake suspensions were supplemented with N+P+K (1:0.5:0.5%, respectively) (Table I), the duration of mycelial growth increased from five to seven weeks on castor; the mycelial growth was initiated in the first week of incubation and the amount also increased to 85% (four and five weeks of incubation) on karanj. Further, N+P+K supplementation to neem cake increased the amount of mycelial growth at all the weekly intervals of observation.

Of the combinations of oil cakes and plant nutrients, the highest mycelial mat weight was recorded uniformly on cake plus N+P+K combinations followed by cake plus N+P and cake plus P combinations (Table II). The mycelial

TABLE I - *Growth of* Verticillium lecanii *on aqueous suspension of oil cakes and their combinations with inorganic fertilizers.*

| Treatment / Weeks | Mycelial growth after incubation (0-100 scale) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| Castor cake (CC) | 40 | 40 | 40 | 40 | 40 | 0 | 0 |
| Karanj cake (KC) | 0 | 20 | 40 | 65 | 65 | 40 | 40 |
| Neem cake (NC) | 40 | 65 | 65 | 80 | 65 | 60 | 60 |
| CC + Phosphorus (P) | 60 | 70 | 80 | 80 | 40 | 0 | 0 |
| KC + P | 0 | 40 | 70 | 80 | 80 | 80 | 60 |
| NC + P | 50 | 70 | 85 | 100 | 100 | 95 | 70 |
| CC + NPK | 55 | 70 | 80 | 80 | 60 | 40 | 20 |
| KC + NPK | 20 | 60 | 60 | 85 | 85 | 60 | 40 |
| NC + NPK | 40 | 65 | 95 | 100 | 100 | 80 | 80 |
| SEM + | 2.54 | 2.88 | 3.31 | 4.40 | 3.44 | 4.33 | 2.55 |
| CD (P = 0.05) | 5.68 | 6.30 | 10.21 | 10.36 | 8.99 | 10.89 | 6.20 |

TABLE II - *Effect of oil cakes and inorganic fertilizers on the mycelial mat weight and spore number of* V. lecanii.

| Treatment | Mycelial mat dry weight (mg) | | | Spore number ($10^4$/ml) | | |
|---|---|---|---|---|---|---|
| | Castor cake | Karanj cake | Neem cake | Castor cake | Karanj cake | Neem cake |
| Cake alone | 90 | 95 | 160 | 8.0 | 13.8 | 19.0 |
| Cake + N (1%) | 30 | 40 | 50 | 6.0 | 8.0 | 11.0 |
| Cake + P (0.5%) | 150 | 155 | 315 | 16.0 | 17.8 | 22.0 |
| Cake + K (0.5%) | 160 | 150 | 295 | 14.0 | 16.0 | 20.0 |
| Cake + NP | 185 | 295 | 296 | 16.0 | 18.0 | 22.0 |
| Cake + NK | 100 | 21 | 55 | 17.0 | 16.0 | 26.0 |
| Cake + PK | 120 | 140 | 282 | 25.0 | 25.8 | 30.0 |
| Cake + NPK | 290 | 325 | 320 | 32.0 | 44.0 | 74.0 |
| SEM + | 3.21 | 2.28 | 3.75 | 0.86 | 1.11 | 1.57 |
| CD (P = 0.05) | 9.29 | 6.63 | 10.87 | 2.50 | 3.24 | 4.56 |

mat weight was lowest in cake plus N combinations followed by cake plus N+K and supplementation of P and K to oil cakes in general have improved the mycelial mat weights either individually (P or K) or in combinations (P+K).

*Verticillium lecanii* sporulated best on neem cake suspension ($19 \times 10^4$ spores/ml) compared to karanj ($13.8 \times 10^4$ spores/ml and castor ($8 \times 10^4$ spores/ml) cakes without any inorganic nutrient supplementation (Table I). Supplementation of the three oil cake suspensions with N+P+K increased sporulation of *V. lecanii* compared to oil cake suspensions without supplementation. Similarly, supplementation with N to all the three oil cakes reduced the sporulation of *V. lecanii* suggesting that the N supplementation created nutritional unbalance in the suspensions.

The natural N, P and K contents in the oil cakes were (i) castor, 4:1.9:1.4%, respectively, (ii) karanj, 4:0.9:1.3% respectively, and (iii) neem, 5.2:1.7:1.5%, respectively.

Out of the three oil cakes, neem has better NPK content which was probably better for the growth and sporulation of *V. lecanii*. Further, supplementation of N, P and K to the oil cake suspensions not only improved the duration and amount of mycelial growth of *V. lecanii* but also enhanced its sporulation. The oil cakes are organic substrates for *V. lecanii* and the inorganic plant nutrients, especially P and K, provided additional nutrients for the growth of the fungus. Therefore, the inorganic fertilizers acted synergistically on the growth of *V. lecanii* on the three organic substrates.

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