# HATCH INHIBITION OF *MELOIDOGYNE INCOGNITA* BY AQUEOUS EXTRACTS AND EXUDATES OF FIVE SPECIES OF CYANOBACTERIA

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**Summary.** The effects of extracts and exudates of the cyanobacteria, autotrophic blue green algae, *Anacystis* (*Synechococcus*) *nidulans*, *Oscillatoria fremyii*, *Lyngbya* sp., *Phormidium molle* and *Westiellopsis prolifica* on hatching of *Meloidogyne incognita* were investigated. Hatch inhibition was greater in extracts than in exudates. The rate of hatch and cumulative percentage hatch over 12 days of incubation were significantly reduced, to 66.9 and 67.6% in exudates (extracellular secretions) of *A. nidulans* and *Lyngbya* sp., respectively, compared to 91.2% hatch in nutrient medium control and 85% in water. After one day of incubation, the rate of hatch in the aqueous extracts (intracellular contents) of the species *A. nidulans* (2.5%), *Lyngbya* sp. (3%) and *O. fremyii* (3.2%) was significantly less that the 29.8% hatch in the control. Cumulative per cent hatch increased to only 7.0, 8.6 and 22.1% over 12 days of incubation compared to 85% in water. Replacing exudates or aqueous extracts with water did not significantly increase the rate of hatch, thus indicating that the suppression of hatch was mostly irreversible.

**Key words:** Blue green algae, *Anacystis* (*Synechococcus*) *nidulans*, *Oscillatoria fremyii*, *Lyngbya* sp., *Phormidium molle* and *Westiellopsis prolifica*.

A vast variety of pests and pathogens cause huge quantitative and qualitative losses of agricultural productivity. The high costs and hazards of pesticides to health and environment have necessitated the search for botanical and microbial bio-pesticides that are expected to be safer. Besides bio-control involving the use of various predators, parasites and pathogens of target pests, search is also on for toxins of microbial origin to replace synthetic pesticides (Varma and Dubey, 1999). Plant parasitic nematodes have been recognized as economically important widespread agricultural pests. Selection and promotion of micro-organisms possessing suppressive or bioregulatory attributes can also play an important role in nematode management. Besides several physical, chemical, biological and cultural methods of control integrated in various ways (Sethi and Gaur, 1986; Bhatti and Walia, 1994; Luc *et al*., 2005), attempts have been made to ascertain the nematicidal or nematostatic properties of cyanobacteria, commonly known as blue green algae. Aqueous exudates (culture filtrates) of *Nostoc muscorum* Agardh ex Bornet *et* Flahault and *Aulosira fertilissima* C.B. Rao significantly reduced the hatch of the rice and wheat root-knot nematode *M. triticoryzae* Gaur, Saha *et* Khan and aqueous extracts inactivated or killed a large proportion of the hatched second stage juveniles (Gaur, 1995). Prior to this, Kumar *et al.* (1993) and Dhanam *et al*. (1994) had reported parasitism of the cyanobacterium *Microcoleus vaginatus* (Vaucher) Gomont ex Gomont in some soil and plant nematodes, but the mechanism of entry and parasitism was not clear. Khan *et al*. (1997) reported that culture filtrates of *M. vaginatus* also suppressed hatch and killed second stage juveniles of *M. incognita* (Kofoid *et* White) Chitw. Youssef and Ali (1998) reported suppression of *M. incognita* infecting cowpea by application of *Anabaena oryzae* (Fritsch) Komarek *et* Anagnostidis, *Nostoc calcicola* Brebisson ex Bornet *et* Flahault and *Spirulina* sp. Sharma *et al.* (2007) have also reported toxicity of ten species of cyanobacteria against *M. incognita*.

The aim of these investigations was to identify more cyanobacteria species showing suppressive effects on root-knot nematode. Therefore, the hatch inhibitory potential of five species of cyanobacteria, viz. *Anacystis* (*Synechococcus*) *nidulans* (Pringscheim) Komarek, *Lyngbya* sp. C. Agardh ex Gomont, *Oscillatoria fremyii* J. de Toni, *Phormidium molle* Kutzing ex Gomont, and *Westiellopsis prolifica* Janet was studied against *M. incognita*.

## MATERIALS AND METHODS

Cultures of the cyanobacteria were obtained from the Center for Conservation and Utilization of Blue Green Algae, Indian Agricultural Research Institute (I.A.R.I.), New Delhi. The cyanobacteria were further cultured on BG-11 medium (Stanier *et al*., 1971) at 28 °C. Live populations of these cultures were used to prepare aqueous extracts (intra-cellular water soluble matter) and exudates (extra-cellular secretions).

*Preparation of aqueous extracts.* The blue green algal mass (100 mg/10 ml) of the cyanobacteria was filtered through Whatman No. 1 filter paper to remove excess medium. The pellet was washed three times with distilled water, ground with a pestle and mortar, suspended in 5

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ml of glass distilled water (GDW) and filtered through Whatman No. 1 filter paper. The filtrate (aqueous extract) was stored at 2 °C until used for hatching tests.

*Preparation of exudates.* The supernatant from the live populations (100 mg/10 ml) of the cyanobacteria was filtered through Whatman No. 1 filter paper. The filtrate (exudate) was stored at 2 °C until used for hatching tests.

*Hatching tests. Meloidogyne incognita* was reared on Pusa-Ruby tomato in a glasshouse at 28 ± 3 °C. When large egg masses had formed, groups of ten of them, each averaging 500 eggs, were put into square glass blocks with hemispherical cavities and incubated in 3 ml of the aqueous extracts or water (control), and 3 ml of exudates, water (control) or nutrient medium (control) at 28 °C for 12 days. Each treatment was replicated three times. The emerging second stage juveniles ($J_2$) were counted after 1, 2, 4, 6, 8 and 12 days and the hatching medium was replaced with fresh at the same time. On the 12$^{th}$ day, the hatching medium of each treatment was replaced with glass distilled water (GDW). Further hatch was recorded on the 14$^{th}$ and 17$^{th}$ days in order to observe any resumption of hatch that occurred after removal of cyanobacterial aqueous extracts and exudates. At the termination of the test, to separate eggs from the gelatinous matrix, 5 ml of 2% NaOCl solution were added to each of the blocks for two hours with mild shaking. The number of embryonated but unhatched eggs was counted and cumulative percentage hatch was computed from the numbers of hatched and unhatched eggs.

The data on percentage hatch were subjected to angular transformation before analysis of variance.

## RESULTS

Emergence of $J_2$ from egg masses of *M. incognita* kept in water was rapid during the first week of incubation. The cumulative hatch increased from 29.8% after one day to 82.2% after 8 days (Table I). Thereafter, the rate of hatch declined gradually to attain a cumulative hatch of 87.9% after 17 days. Egg hatch in the nutrient medium control was significantly more than that in the water control as 34.6% of eggs hatched after one day, 88.6% after 8 days, 91.2% after 12 days and continued, although slowly, even after shifting the egg masses to water to reach 93.2% and 93.5% cumulative hatch after 14 and 17 days (Table II).

*Hatching in aqueous extract.* After 1 day, hatch in aqueous extract of *A. nidulans* (2.5%), *Lyngbya* sp. (3.0%) and *O. fremyii* (3.2%) was significantly suppressed compared to that in the water control (29.8%). Hatch of eggs in aqueous extracts of the other two species, viz., *W. prolifica* (30.7%) and *P. molle* (26.7%), was similar to that in water. More or less similar hatching trends were observed up to 6 days. After 8 days there was very little hatch in aqueous extracts of *A. nidulans* and *Lyngbya* sp., while egg hatch in the aqueous extract of *O. fremyii* was inhibited only up to 6 days (7.5%) and increased to 20% after 8 days and to 22.1% after 12 days. Egg hatch continued at similar rates up to 8 days in aqueous extract of *W. prolifica* (80.4%) and *P. molle* (73.4%) and was at par compared with that in water (82.2%). A similar pattern was observed on the 12$^{th}$ day, when the cumulative hatch was only 7.0% and 8.6% in aqueous extract of *A. nidulans* and *Lyngbya* sp., respectively, compared with the 22.1%, 76.0%, 82.7% in aqueous extract of *O. fremyii, P. molle* and *W. prolifica*, respectively, and 85% in the water control.

Transferring the egg masses to water after 12 days resulted in only 0.1-7.1% increase in cumulative hatch for all extracts. Evidence of a small resumption of hatch was observed for the extracts of *O. fremyii* and *P. molle*, as 6% and 7.1% hatch, respectively, occurred during the first 2 days of transfer to water.

*Hatching in exudates.* During the first day of incubation in exudate of *A. nidulans*, hatch (26.5%) was slightly less than that in water, while the hatch in exudates of the other four species, viz., *W. prolifica* (31.3%), *P. molle* (31.4%), *O. fremyii* (29.2%) and *Lyngbya* sp. (29.1%) was at par with that in water but significantly lower than that in the nutrient medium (34.6%) (Table II). More or less similar hatch trends were observed up to 4 days. After 6 days, hatch was negligible in exudates of *A. nidulans* and *Lyngbya* sp. but, up to 8 days, hatch in exudates of *W. prolifica* (82.2%), *P. molle* (84.8%) and *O. fremyii* (80.2%) was similar to those in water (82.2 %) and in nutrient medium (88.6%) controls. On the12$^{th}$ day, cumulative hatches were 66.9% and 67.6% in exudates of *A. nidulans* and *Lyngbya* sp., respectively, compared with the 83.4-86.1% hatch in exudates of the other three cyanobacteria species and the 85 and 91.2% hatch in water and nu-

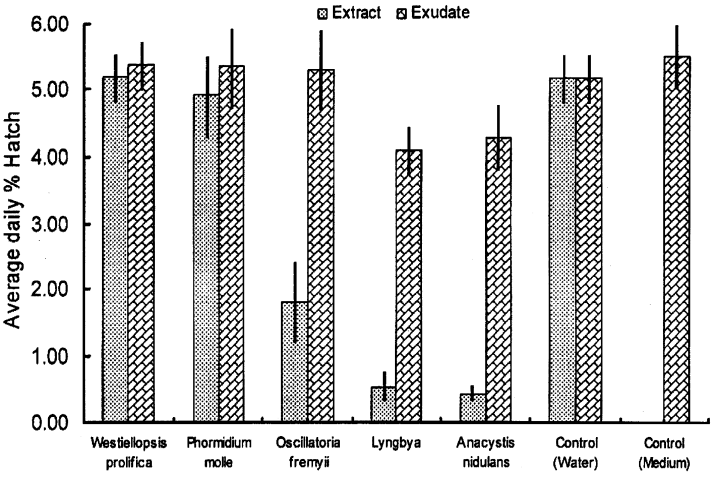


**Fig. 1.** Effect of aqueous extracts and exudates of five species of cyanobacteria on the average daily percentage hatch of *Meloidogyne incognita.*

**Table I.** Effect of aqueous extracts of five species of cyanobacteria on cumulative % hatch of *Meloidogyne incognita*.

| Cyanobacteria | Day –1 | Day –2 | Day –4 | Day –6 | Day –8 | Day –12 | Day –14 in water | Day –17 in water |
|---|---|---|---|---|---|---|---|---|
| *Westiellopsis prolifica* | 30.7 | 48.1 | 61.3 | 70.6 | 80.4 | 82.7 | 87.2 | 88.0 |
| | (33.7)a | (43.9)a | (51.6)a | (57.2)a | (63.7)a | (65.4)b | (69.0)a | (69.7)a |
| *Phormidium molle* | 26.7 | 41.2 | 52.9 | 62.6 | 73.4 | 76.0 | 83.1 | 83.8 |
| | (31.1)b | (39.9)b | (46.6)c | (52.3)b | (59.0)b | (60.7)c | (65.8)b | (66.3)b |
| *Oscillatoria fremyii* | 3.2 | 5.2 | 6.5 | 7.5 | 20.0 | 22.1 | 28.1 | 30.3 |
| | (10.4)c | (13.2)c | (14.7)d | (15.9)cd | (26.5)c | (28.1)d | (32.0)c | (33.4)c |
| *Lyngbya* sp. | 3.0 | 4.5 | 5.9 | 7.7 | 8.5 | 8.6 | 8.7 | 8.9 |
| | (9.9)c | (12.2)cd | (14.1)de | (16.1)c | (16.9)d | (17.0)e | (17.2)d | (17.4)d |
| *Anacystis nidulans* | 2.5 | 3.7 | 4.7 | 6.1 | 6.9 | 7.0 | 7.3 | 7.4 |
| | (9.0)c | (11.0)d | (12.6)e | (14.3)d | (15.2)d | (15.4)f | (15.7)e | (15.8)d |
| Control (Water) | 29.8 | 47.0 | 57.9 | 69.6 | 82.2 | 85.0 | 87.6 | 87.9 |
| | (33.1)ab | (43.3)a | (49.5)b | (56.5)a | (65.1)a | (67.2)a | (69.3)a | (69.6)a |
| CD (P<0.05) | 2.14 | 1.68 | 1.67 | 1.75 | 1.80 | 1.54 | 1.34 | 1.64 |

Figures in parentheses are angular transformed values of percentages.
Figures flanked by the same letter of the alphabet are statistically at par at $P < 0.05$.

**Table II.** Effect of exudates of five species of cyanobacteria on cumulative % hatch of *M. incognita.*

| Cyanobacteria | Day –1 | Day –2 | Day –4 | Day –6 | Day –8 | Day –12 | Day –14 in water | Day –17 in water |
|---|---|---|---|---|---|---|---|---|
| *Westiellopsis prolifica* | 31.3 | 47.1 | 62.9 | 69.3 | 82.2 | 83.4 | 86.9 | 91.3 |
| | (33.90)b | (43.11)c | (52.24)b | (56.07)c | (64.68)c | (65.51)d | (68.32)c | (72.95)$^{b}$ |
| *Phormidium molle* | 31.4 | 49.2 | 64.4 | 80.0 | 84.8 | 86.1 | 90.2 | 91.1 |
| | (34.05)b | (44.54)b | (53.37)b | (63.44)b | (67.05)b | (68.07)b | (71.76)b | (72.64)$^{bc}$ |
| *Oscillatoria fremyii* | 29.2 | 45.5 | 58.7 | 71.3 | 80.2 | 83.4 | 89.6 | 89.9 |
| | (32.71)b | (42.42)c | (50.01)c | (57.61)c | (63.58)c | (65.92)cd | (71.14)b | (71.47)$^{c}$ |
| *Lyngbya* sp. | 29.1 | 45.4 | 58.4 | 64.0 | 65.4 | 67.6 | 69.0 | 70.0 |
| | (32.65)b | (42.36)c | (49.83)c | (53.10)d | (53.94)d | (55.28)e | (56.17)e | (56.78)$^{f}$ |
| *Anacystis nidulans* | 26.5 | 40.3 | 53.6 | 62.2 | 65.4 | 66.9 | 72.6 | 73.0 |
| | (30.98)c | (39.43)d | (47.03)d | (52.07)d | (53.97)d | (54.90)e | (58.40)d | (58.69)$^{e}$ |
| Control (Water) | 29.8 | 47.0 | 57.9 | 69.6 | 82.2 | 85.0 | 87.6 | 87.9 |
| | (33.08)b | (43.28)bc | (49.52)c | (56.53)c | (65.05)c | (67.18)bc | (69.35)c | (69.65)$^{d}$ |
| Control (Nutrient | 34.6 | 52.7 | 70.4 | 82.9 | 88.6 | 91.2 | 93.2 | 93.5 |
| Medium) | (36.00)a | (46.55)a | (57.01)a | (65.56)a | (70.24)a | (72.71)a | (74.85)a | (75.25)$^{a}$ |
| CD (P<0.05) | 1.331 | 1.293 | 1.623 | 2.005 | 1.593 | 1.465 | 1.346 | 1.233 |

Figures in parentheses are angular transformed values of percentages.
Figures flanked by the same letter of the alphabet are statistically at par at $P < 0.05$.

trient medium controls, respectively. Transferring the egg masses to water after 12 days resulted in only 1.4-6.2% cumulative egg hatch increase for all the species of cyanobacteria, similar to that in the two controls (2.2-2.6%). The greatest, but still small, resumption of hatch during the 2 days after transfer to water occurred in egg masses that had been incubated in exudates of *A. nidulans* (5.7%) and *O. fremyii* (6.2%).

A comparison of the average daily hatch in the different treatments (Fig. 1) indicates that aqueous extracts and exudates of *A. nidulans* and *Lyngbya* sp. caused strong hatch inhibition followed by *O. fremyii*, while *P. molle* and *W. prolifica* did not cause significant hatch reduction.

## DISCUSSION

The aqueous extracts of *A. nidulans*, *Lyngbya* sp. and *O. fremyii* caused an immediate and strong inhibition of hatching. The inhibition was evident within one day of incubation and less than 5% hatch occurred in the extracts of these three species up to 6 days compared to 68.6% in water. The inhibition in extracts of *A. nidulans* and *Lyngbya* sp. continued up to 12 days, but about 12% hatch occurred during the 6-8$^{th}$ day period in the case of *O. fremyii* without further increase up to 12 days. It is difficult to explain the sudden increase in hatch in *O. fremyii* during the 6-8$^{th}$ day period only. It is possible that the inhibitory factor in *O. fremyii* was not

stable beyond one week, but if this were so more hatch would be expected beyond the 8th day.

The hatching of *M. incognita* was characteristically rapid in the water and nutrient medium controls compared to that in cyanobacterial exudates. Also, the nutrient medium stimulated more hatch than the water control, thus indicating that it stimulates hatch by itself or that it did not cause any inhibition of hatch.

About 25-30% reduction of total hatch was caused by exudates of *A. nidulans* and *Lyngbya* sp. These findings conform to those of Gaur (1995) for *N. muscorum* and *A. fertilissima*, Khan *et al*. (1997) for *M. vaginatus* and Youssef and Ali (1998) for *A. oryzae, N. calicola* and *Spirulina* sp. The exudates of *O. fremyii, P. molle* and *W. prolifica*, possessing relatively less effective and inhibitory factor(s), were not stable beyond one week of storage at 2 °C. In general, the hatch inhibition due to exudates was mild as substantial hatch (up to 65%) could occur even with the most effective species, *A. nidulans*.

These data thus showed that, at the concentration tested, hatch inhibition by aqueous extracts of *A. nidulans*, *Lyngbya* sp. and *O. fremyii* and exudates of *A. nidulans* and *Lyngbya* sp. was mostly irreversible, as even after 4 days of transfer to water the cumulative per cent hatches were significantly smaller than in the control.

Whapham *et al.* (1994) reported that the numbers of eggs of *M. javanica* (Treub) Chitw. produced on plants treated with extract of the seaweed *Ascophilum nodosum* Le Jolis were reduced after one generation and that the fecundity of the females developed from the $J_2$ hatched in the extracts of *A. nodosum* was also reduced. It appears that the cyanobacteria contain factors which inactivate the $J_2$ both within and outside the egg, thus resulting in the immobility or death of the hatched $J_2$.

The extracts of several marine algae have already been reported to suppress nematode infection and reproduction (Pracer *et al.*, 1987; Tarjan *et al.*, 1988; De Waele *et al.*, 1988; Whapham *et al.*, 1994). Dried algal catch from an inland waterbody has also been found to reduce nematode infection (Kaul and Chhabra, 1993).

De Waele *et al.* (1988) found that the highest concentration that they prepared of an extract of the brown kelp, *Ecklonia maxima* (Osbeck) Papenfuss, suppressed the reproduction of *Pratylenchus zeae* Graham but also weakened the maize plants that they used, rendering them more intolerant of nematode attack. Microbial blue green algae and all other tested products significantly reduced the numbers of root-knot nematode juveniles in soil, females, egg masses, the rate of population build-up, gall formation on roots and significantly increased the plant growth parameters (Ismail and Hasabo, 2000).

Our results have shown that the five species of cyanobacteria possess factors in their cytoplasm as well as secretions that could inhibit hatching of nematode eggs. Also it appears that hatch inhibiting factors from *A. nidulans* and *Lyngbya* sp. were relatively stronger than those from the other three species tested.

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