EFFECT OF NEEM CAKE ON THE POPULATION AND NITROGEN FIXING ACTIVITY OF BLUE-GREEN ALGAE IN FLOODED SOIL

I. WATANABE, B. P. R. SUBUDHI AND T. AZIZ

Soil Microbiology Department, International Rice Research Institute, Los Baños, Laguna, Philippines

ABSTRACT

Neem cake stimulated the growth of blue-green algae in flooded soil possibly by depressing the activity of grazers, and enhanced photodependent N_2-fixation. Neem cake lessened the depressing action of ammonium to the population of blue-green algae and photodependent N_2-fixation. These effects were first observed in the greenhouse, and a similar trend was found in the field.

INTRODUCTION

INSECT repellent action of neem cake (deoiled, crushed seed kernel of neem *Azadirachta indica*) has been established because of the presence of terpenoid substances like Azadirachtin. Reports indicate that neem cake reduces the loss of N-fertilizers by inhibiting the population of nitrifiers which are the main cause of *N* loss due to nitrification of NH_4–N in the flooded soil[1–3]. Neem cake, when blended with urea, increases the uptake of N, P and K[4,5] and enhances the yield of rice[6,7]. Reports also state that the growth of blue-green algae (BGA) is promoted by the application of insecticide carbofuran, possibly by killing small animals or grazers that eat BGA and make the water turbid[8]. Because neem cake has an insecticidal effect, it may also promote the growth of BGA and photodependent N_2-fixation in flooded soil. The present investigation deals with the effects of neem cake on BGA and their nitrogen fixation.

MATERIALS AND METHODS

A chemical analysis of the well ground neem cake sample was: 22% moisture, 10·6% ash, 11·6% crude protein, 4·9% crude fat, 1·5% K, 0·39% Mg, 0·33% Ca, 0·15% Fe, and 0·004% Zn.

Experiments were conducted in 4 replications in the greenhouse in beakers, and in 3 replications in the field. Greenhouse experiments were : control, 57 kg neem cake/ha, 60 kg N/ha + 57 kg neem cake/ha and 60 kg N (ammonium sulphate)/ha + 114 kg neem cake on surface area basis. The field treatments were: control, 114 kg neem cake/ha, 120 kg N/ha, 120 kg N/ha + 114 kg neem cake/ha and 120 kg N + 67 *l* neem oil/ha. Fresh soil (250 gm) was taken in each of the 250 ml pyrex beakers. Fertilizers and neem cakes were mixed thoroughly in the surface soil up to 3 cm depth. For the field experiment, plot sizes were 3 m × 4 m, transplanted with 16-day-old seedlings of IR1917-3-17, with 20 cm × 20 cm spacing. Fertilizers and neem cake were mixed thoroughly up to 20 cm depth one day before transplanting. Water level was maintained at 3 cm every day.

To determine phototrophic acetylene reduction activity (ARA), 7 core samples (1·9 cm diameter) with 3 cm depth soil along with the surface water were collected from each plot. The tubes were put in polyethylene bags (30 cm × 23 cm) with a rubber septa and sealed. After evacuation, a known volume of air with 10% acetylene was injected and incubated in the phytotron at 40 klux and 25° C for 1 hour, then the ethylene was analyzed by gas chromatography. For ARA in beakers, the entire beakers were placed into plastic bags and treated the same way as ARA determination from the field using the core samples.

BGA field counts were made from the soil core sample which was used to determine ARA. But, for the algal counts from the beakers, four core samples with 1 cm depth soil were collected from each beaker and passed through a 1 mm sieve. Ten fold dilutions were made and inoculated in the media of Allen and Arnon[9] then grown under 200 lux for 2 weeks. Fresh weights of algal biomass from control and neem cake treated plots also were taken to assess BGA growth in the field. Algal biomass was determined by taking its fresh weight in 400 cm^2 area using a metal frame of that size. Ten such areas were selected randomly from each plot to get an average value.

RESULTS AND DISCUSSION

Experiments in the beakers (Table I) show that neem cake increased the population of BGA over the control, particularly at 25 and 35 days. At 15 days, the population in ammonium treated beakers was depressed, followed by gradual increase. Neem cake initially appeared to lessen the depressing effect of ammonium nitrogen. Table II shows the changes of ARA under light. The neem cake treatment increased N_2 fixation in non-nitrogen treated beakers and maintained high activity after 24 days, when the activity in the control declined. ARA under dark was less. The addition of ammonium depressed photodependent N_2 fixation in the floodwater and at the soil surface. The activity was gradually regained.

TABLE I

Effect of neem cake on blue-green algal population (in beakers with soil from IRRI, block 900)

Treatment/ha	Log number of cells. gm^{-1} dry soil — Mean ± standard deviation (4) replicates		
	Days after application		
	15	25	35
Control	3·92±0·15	4·31±0·02	4·29±0·18
57 kg Neem cake	4·23±0·05	5·13±0·04	4·99±0·01
60 kg N*	3·10±0·17	4·05±0·18	4·72±0·05
60 kg N + 57 kg Neem cake	3·86±0·07	4·53±0·05	4·70±0·13
60 kg N+114 kg Neem cake	3·76±0·15	4·31±0·05	4·75±0·13

* N as ammonium sulphate.

algae, BGA developed in the control and in the neem cake treated plots. At the 9th week after transplanting, 2 of the 3 neem cake treated plots had algal biomass higher than 2·5 tons fresh weight/ha, while 2 of the 3 control plots recorded algal biomass less than 100 kg fresh weight/ha. Thereafter, algal biomass increased in both the control and neem treated plots. Algal biomass was always higher in neem cake treated plots than in the control plots of the same blocks. The ARA in neem cake treated plots was slightly higher than that of the control plots at the 12th and 15th weeks (Table IV).

Although the field experiment did not show stimulating effects of neem cake so markedly as the beaker experiment, a similar trend in the beaker was observed in the field. In the paddy field, the development of BGA was probably determined by unknown or uncontrolled factors.

The stimulating effect of neem cake to photodependent N_2 fixation was never studied.

TABLE II

Effect of neem cake on N_2-fixation in soil (experiment conducted in beakers with soil from IRRI field, block 900)

Treatment/ha	Log of μ mole $C_2H_4 \cdot hr^{-1} \cdot m^3$ — Days after application				
	10	17	24	31	45
Control	0·74±0·09	2·52±0·17	2·73±0·16	1·54±0·11	0·93±0·26
57 kg NC	1·69±0·15	2·96±0·13	3·04±0·1	3·09±0·02	2·59±0·55
N*	0·35±0·27	0·87±0·11	2·19±0·04	2·70±0·11	2·63±0·78
N+ 57 kg NC	0·70±0·29	2·57±0·12	2·94±0·15	2·70±0·07	2·47±0·35
N + 114 kg NC	0·15±0·13	2·29±0·1	2·41±0·09	2·81±0·13	2·79±0·16

* N = 60 kg N or 285 kg ammonium sulphate. NC = Neem cake.

The addition of neem cake also lessened the depressing action of ammonium to photodependent N_2 fixation until 24 days. The decline of photodependent N_2 fixation in control beakers at about 30 days was visually accompanied by the decline of algal scum which was composed of *Anabaena* cells. Algal cells were actively digested by ostracods, while in the neem treated beakers, the digestion and the activity of ostracods were less intense and started only at about 40 days.

Experiments using another soil from the International Rice Research Institute (IRRI) field (Table III) showed the stimulating effect of neem cake to N_2 fixation particularly during the early incubation, although the effect was not so marked as the previous experiment (Table II). The effect of neem cake was not clear in ammonium treated beakers.

Heavy rain in the field experiment retarded algal growth by the 5th week after transplanting. After the development of diatoms and filamentous green

TABLE III

Effect of neem cake on N_2-fixation in soil (experiment conducted in beakers with soil from IRRI field, upper MN)

Treatment/ha	Log of μmole $C_2H_4 \cdot hr^{-1} \cdot m^{-2}$ — Days after application		
	20	40	50
Control	1·61±0·69	2·11±0·14	2·46±0·16
N*	1·42±0·06	1·48±0·10	1·39±0·04
114 kg N Neem cake	2·30±0·12	2·38±0·12	2·44±0·11
228 kg Neem cake	2·18±0·08	2·50±0·21	2·42±0·23
N + 114 kg Neem cake	1·56±0·03	1·60±0·05	1·39±0·03

*N = 120 kg N or 571 kg ammonium sulphate.

TABLE IV

Effect of neem cake on nitrogen fixation in field (IRRI rice field, upper MN)

Treatment/ha	Log of μmole C_2H_4 hr^{-1} m^{-2}		
	Weeks after transplanting		
	9	12	15
Control	1·67±0·13	1·63±0·25	1·17±0·30
114 kg Neem cake	1·80±0·25	2·05±0·34	2·10±0·24
N*	1·45±0·13	1·53±0·06	1·39±0·17
N + 114 kg Neem cake	1·60±0·1	1·58±0·05	1·77±0·34
N + 67 *l* Neem oil	1·50±0·07	1·58±0·03	1·34±0·04

*N = 120 kg N or 571 kg ammonium sulphate.

Neem cake may exert its action to BGA specifically and non-specifically. The depression of grazers of BGA is the specific action. The neem cake may have a non-specific effect as other organic matter such as rice crop residues. The organic matter immobilizes ammonium, absorbs O_2 and releases CO_2, and provides physical support for BGA. These effects are all favourable to the growth of BGA. The growth of *Anabaena* in liquid culture in the laboratory was neither inhibited nor stimulated by the level of neem cake applied in the pot.

It is suspected that specific action-depression of the grazers is more important, because the amount applied is less than those generally given by crop residues.

ACKNOWLEDGEMENT

The authors acknowledge Dr. R. C. Saxena at the International Rice Research Institute (IRRI) for his encouragement and advice. Also, Mr. C. M. Ketkar, Non-Edible Oils and Soap Industry, Puna, India, for providing neem cakes.

1. Bains, S. N., Prasad, R. and Bhatia, P. C., *Fertil. News*, 1971, **16** (March), 30.
2. Reddy, R. N. S. and Prasad, R., *J. Soil Sci.*, 1975, **26**, 304.
3. Subbiah, S. and Kothandaraman, G. V., *J. Indian Soc. Soil Sci.*, 1980, **28**, 136.
4. Harisharkar, Babu Ram and Rathi, K. S., *Ibid.*, 1976, **24**, 211.
5. Sinha, N. P., Singh, S. N. and Jha, B. K., *Indian J. Agric. Sci.*, 1979, **49**, 272.
6. Ketkar, C. M., *Fertil. News*, 1974, **19** (Feb.), 25.
7. Subbiah, S., *Madras Agric. J.*, 1976, **66**, 678.
8. Tirol, A. C., Santiago, S. T. and Watanabe, I., *J. Pesticide Sci.*, 1981, **6**, 21.
9. Allen, M. B. and Arnon, D. I., *Plant Physiol.*, 1955, **30**, 366.

GABA INDUCED NEUROSECRETION IN THE COCKROACH

N. K. MISHRA

Department of Zoology, Patna University, Patna 800 005, *India*

AND

APARAJITA SRIVASTAVA

Government Girls College, Gulzarbagh, Patna, India

ABSTRACT

Neurosecretory (N.S.) cells of the ventral ganglia of the female cockroach, *Periplaneta americana*, have been examined following supplemental feeding with 1% gamma amirobutyric acid (GABA). Karyometry of the n.s. cells and analysis of secretory material reveal their enhanced activity in 1st and 3rd thoracic, and in 1st, 2nd and 4th abdominal ganglia. This activity coincides with charges in tissue protein, glycogen and phospholipid. In the ovary, glycogen and phospholipid contents increase. In the fat body, while protein concentration is lowered, glycogen content is increased.

INTRODUCTION

GAMMA aminobutyric (GABA) acid is of great interest in the nervous tissue of vertebrates[1]. Injection of GABA in the third ventricle of male rats promotes the release of luteinizing hormone. GABA probably controls the discharge of hypothalamic lutienizing hormone releasing factor[2]. In the honey bee brain, L-glutamic acid decarboxylase is inhibited by high concentration of GABA which in turn can be