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NITRIFICATION INHIBITORS
I. STUDIES WITH KARANJIN, A FURANOLFLAVONOID FROM KARANJA (*PONGAMIA GLABRA*) SEEDS

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SUMMARY

Karanjin, the major crystalline principle of karanja (*Pongamia glabra* Vent.) seeds, is shown to be a potent nitrification inhibitor. It compares well with N-serve in pot-culture experiments with rice crop. Structure activity relationship studies with chemically altered molecules from karanjin show that its furan ring is essential for showing the inhibitory effect.

INTRODUCTION

It is now well recognized that in the tropics only 25–40 per cent of nitrogen applied in the form of fertilizers, is utilized by plants. The losses are due to bacterial denitrification and leaching. This has assumed a greater significance in the present context of worldwide nitrogen fertilizer shortage and their soaring costs. In upland agriculture, leaching of nitrified nitrogen is the major pathway of nitrogen loss, while under submerged conditions, denitrification seems to be the principal mode. Current concern all over the world is, therefore to find methods by which the process of nitrification can be suitably controlled. Application of certain chemicals called nitrification inhibitors seems to be the easiest short-term approach towards this problem. Several chemicals like N-serve, AM *etc.*, have

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come into prominence in this context and the agronomic aspects of this approach have been the subject of many reviews[1 8].

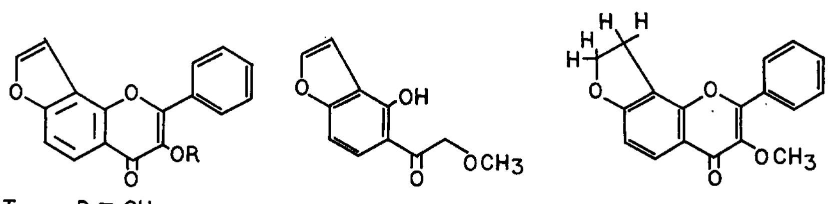
In India, non-edible oil seed cakes like karanja (*Pongamia glabra*) and neem (*Azadirachta indica*) have been used since time immemorial in admixture with manures with adavantages. Beneficial effects of these cakes have been attributed to their nitrification inhibitory properties. Several workers have tried to establish this by carrying out controlled experiments in laboratory as well as greenhouse using crude extracts of these cakes[14 15]. Sahrawat *et al.*[11] extended this work to find the efficacy of extracts of seed, bark and leaves of karanja tree. In this paper we describe the nitrification inhibitory property of karanjin, the principal crystalline chemical constituent of karanja seed, which is responsible for this activity. Further by suitable alterations of the molecule and studying the effect on this inhibitory property, it is shown that the furan ring present in the molecule seems to be the crucial structural factor for this activity.

MATERIALS AND METHODS

A. *Test inhibitors*

Karanjin (3-methoxy furano-2′,3′,7,8-flavone; I) was isolated from karanja seeds and oil[10]. Karanj ketone (4-hydroxy-5-ω-methoxyacetyl coumarone; II) and karanjonol (3-hydroxy furano-2′,3′,7,8-flavone; III) were prepared from karanjin[5].

Dihydrokaranjin (3-methoxy dihydrofurano-2′,3′,7,8-flavone; IV) prepared by hydrogenation of karanjin, was obtained as colourless needles (m.p. 187°C) from ethanol (Found C, 73.4; H, 4.5, $C_{18}H_{14}O_4$ requires C, 73.5; H, 4.7%). NMR ($CDCl_3$, δ): 3.30 (2P, t, J = 8.5 Hz, β-CH_2), 3.38 (3P, s, OCH_3), 4.76 (2P, t, J = 8.5 Hz, α-CH_2), 6.80 (1P, d, J = 8.5 Hz, H_6), 7.50 (5P, m, C_6H_5), 8.05 (1P, d, J = 8.5 Hz, H_5).



I, R = CH_3

III, R = H

II

IV

Structural formulas of the test inhibitors: – I, karanjin; II, karanj ketone; III, karanjonol; and IV, dihydrokaranjin.

'N-serve' (2-chloro-6-trichloro-methyl pyridine), obtained from Dow Chemical Company, was used as reference inhibitor.

B. *Soil*

Sandy loam soil from the Institute's farm, (pH: 7.7; Organic carbon: 0.58% CEC: 8.9 meq/100 g; water holding capacity: 31.7%) was used for laboratory and pot experiments.

C. *Fertilizers*

Ammonium sulphate and urea were used as sources of nitrogen. Single superphosphate, (16% P_2O_5) and potassium sulphate (54% K_2O) were used to supply P and K in pots.

DESCRIPTION OF EXPERIMENTS AND METHODS

A. *Laboratory incubation studies*

Karanjin (I) and its derived products (II, III and IV) were screened as nitrification inhibitors at 5, 10 and 20% concentration (NH_4^+–N basis) using 200 ppm N from both the fertilizers. The treated soils were incubated at 30°C at 1/3 water holding capacity. Soil samples were drawn at 15, 45 and 75 days, extracted with Morgan's reagent[12], and analysed for NH_4^+-[13] and NO_2^-- and NO_3^--nitrogen[9].

Karanjin and N-serve were, then, compared at one concentration (5%, NH_4^+–N basis) using both nitrogen sources at 200 ppm N-level by drawing samples at weekly intervals.

B. *Pot-culture experiments*

Karanjin was compared with 'N-serve' at 5, 10 and 15 per cent doses (NH_4^+–N basis) using both the fertilizers as nitrogen sources at the rate of 150 kg N/ha along with basal doses of 60 kg/ha of each of P and K. Acetone solution of the inhibitors and aqueous solution of nitrogen fertilizers were thoroughly mixed with the soil and filled in 10 kg capacity pots. Three week old seedlings of 'Bala' variety of rice were transplanted in these pots and maintained under flooded conditions. On maturity, the yield of grain and straw were recorded, analysed for nitrogen and nitrogen uptake calculated. The data were statistically analysed.

RESULTS AND DISCUSSION

A. *Laboratory incubation experiments*

The results obtained in these experiments with karanjin, karanj ketone and dihydrokaranjin at 5, 10 and 20 per cent (N-dose basis) levels are given in Table 1. Their various effects are discussed below:

TABLE 1

Effect of test inhibitors on production of ammonical, nitrite and nitrate nitrogen in incubated soil after 15, 45 and 75 days*

N-source	Inhibitor (% N basis)	NH_4^-–N (ppm)			NO_2^-–N (ppm)			NO_3^-–N (ppm)		
		15	45	75	15	45	75	15	45	75
	Karanjin									
Ammonium sulphate	0	95	33	10	1.0	Tr.	—	18	90	155
	5	160	70	17	1.7	1.1	—	8	44	101
	10	171	86	25	2.2	2.7	0.5	7	32	90
	20	181	110	33	2.2	3.3	0.6	6	25	83
Urea	0	100	31	11	0.9	0.3	—	15	94	160
	5	169	70	18	2.0	1.0	—	8	45	91
	10	173	81	27	2.2	2.7	0.6	8	35	88
	20	188	105	35	2.3	3.0	0.5	6	25	79
	Karanj ketone									
Ammonium sulphate	0	112	29	16	0.6	Tr.	—	20	91	145
	5	129	39	15	0.6	Tr.	—	18	89	140
	10	167	83	18	1.2	Tr.	—	7	87	138
	20	178	89	16	0.6	Tr.	—	6	50	131
Urea	0	86	32	10	0.6	Tr.	—	18	89	142
	5	136	43	11	0.6	Tr.	—	9	88	140
	10	142	53	16	1.2	Tr.	—	8	86	142
	20	150	60	20	3.3	Tr.	—	4	46	144
	Karanjonol									
Ammonium sulphate	0	119	30	16	Tr.	Tr.	—	20	91	145
	5	141	39	16	6.4	Tr.	—	17	80	140
	10	155	46	18	6.4	Tr.	—	9	81	135
	20	175	80	24	2.3	Tr.	—	7	58	130
Urea	0	85	31	9	Tr.	Tr.	—	18	88	143
	5	111	40	9	2.3	Tr.	—	11	88	145
	10	117	46	12	3.3	Tr.	—	8	83	141
	20	134	61	10	2.3	0.6	—	6	56	138
	Dihydrokaranjin									
Ammonium sulphate	0	113	31	16	0.6	Tr.	—	20	91	145
	5	119	30	15	2.3	Tr.	—	18	91	147
	10	129	30	15	1.2	Tr.	—	12	90	146
	20	130	35	15	0.6	Tr.	—	10	84	145
Urea	0	85	31	12	Tr.	Tr.	—	20	89	144
	5	85	31	10	0.6	Tr.	—	19	89	144
	10	86	31	9	1.2	Tr.	—	17	89	144
	20	119	35	11	2.3	Tr.	—	10	86	140

* Oven dry soil weight basis; N-dose = 200 ppm; (—) denotes undetectable; Tr. = Traces.

1. Conservation of ammoniacal nitrogen. It is seen from Table 1 that excepting dihydrokaranjin, all the other test chemicals are effective inhibitors of nitrification and in all cases the effect increased and lasted longer with increased concentration. Karanjin is the most effective inhibitor, followed by karanj ketone and karanjonol.

2. Production of nitrite and nitrate nitrogen. In general, none of the test chemicals, at the levels tested, showed any significant nitrite accumulation, irrespective of the source of nitrogen.

Nitrate production was reduced most in fertilizers treated with karanjin at 20 per cent level. The nitrate formation improved with time in all the treatments. The regulatory effect lasted with all chemicals upto 75 days and karanjin remained effective beyond this period even at 5 per cent dosage.

3. Comparison of karanjin and N-serve. The results of comparison of the nitrification inhibitory effect of karanjin and N-serve are given in Figures 1 and 2. Both these chemicals were tested at 5 per cent (NH_4^+–N-basis) concentration. Maximum

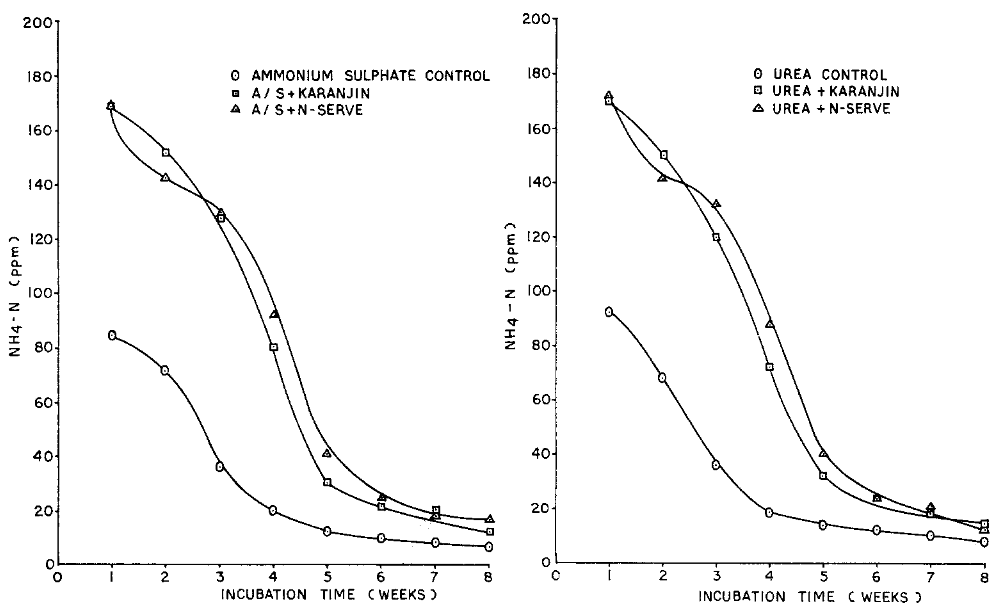


Fig. 1. Effect of Karanjin and N-serve on production of ammonical nitrogen in incubated soils.

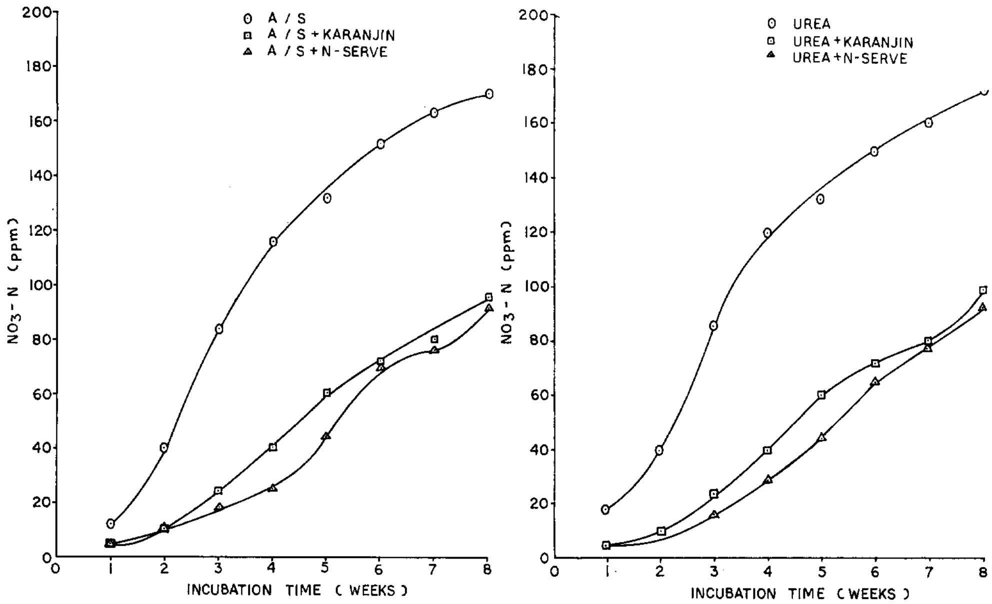


Fig. 2. Effect of Karanjin and N-serve on production of nitrate nitrogen in incubated soils.

accumulation of ammoniacal nitrogen was observed after first week of incubation and both showed comparable results with ammonium sulphate as well as urea, Ammoniacal nitrogen was almost doubled as compared to control. However, after five weeks, the performance of N-serve was slightly better than karanjin. Both 'N-serve' and karanjin treated soils had higher amounts of NH_4^+–N than control even after 8 weeks – the duration period of these studies.

Karanjin as well as 'N-serve' did not show any appreciable amount of nitrite accumulation, indicating that both chemicals did not inhibit the nitrite oxidising bacteria, Nitrobactor sp. Such results have been reported for 'N-serve' by many workers[2 3 6 16], and for the crude extracts of karanja cake by Singh[14]. Nitrate production was also considerably reduced by the two compounds, maximum inhibition being after first week. Thereafter, the effect decreased with time presumably with the re-establishment of the population of nitrifying organisms.

B. *Pot-culture experiments*

The data obtained with both karanjin and N-serve inhibitors from pot-culture experiments were statistically analysed and are given in Tables 2 and 3.

1. Dry matter production (grain + straw). The addition of N-serve or karanjin significantly improved the dry matter yield in all cases. No significant difference between the various levels of inhibitors treatments was observed with both the fertilizers except

TABLE 2

Mean yield (g/pot) of grain and grain + straw of rice as affected by karanjin and N-serve in combination with ammonium sulphate and urea fertilizers

Treatment	Ammonium sulphate		Urea	
	Grain	Grain + straw	Grain	Grain + straw
Fertilizer alone	27.3	64.3	29.3	65.7
+5% Karanjin	36.0	78.0	40.7	79.4
+5% N-serve	36.0	76.3	36.0	78.0
+10% Karanjin	42.0	79.3	41.0	75.7
+10% N-serve	41.0	81.0	40.0	73.7
+15% Karanjin	40.3	74.3	41.0	82.3
+15% N-serve	36.0	74.7	23.3	48.3
'F' Test	Sign.	Sign.	Sign.	Sign.
SE (m)	1.68	3.56	2.24	3.07
CD (5%)	3.66	7.76	4.88	6.69

TABLE 3

Mean nitrogen uptake (mg N/pot) by grain and grain + straw of rice as affected by karanjin and N-serve in combination with ammonium sulphate and urea fertilizers

Treatment	Ammonium sulphate		Urea	
	Grain	Grain + straw	Grain	Grain + straw
Fertilizer alone	339.1	503.4	359.7	513.0
+5% Karanjin	460.9	679.9	508.3	701.5
+5% N-serve	426.9	601.0	433.4	638.5
+10% Karanjin	552.5	744.6	538.3	704.8
+10% N-serve	535.6	727.6	546.1	716.4
+15% Karanjin	570.7	740.6	560.8	781.3
+15% N-serve	463.5	652.0	282.6	408.2
'F' test	Sign.	Sign.	Sign.	Sign.
SE (m)	29.96	23.24	51.90	30.85
CD (5%)	65.28	50.64	113.09	67.22

the ·15 per cent N-serve treatment with urea as nitrogen source which significantly lowered the yield.

2. Grain yield. All treatments involving karanjin with both the fertilizers gave significantly higher grain yield compared to the fertilizer control. Similarly, all levels of 'N-serve' except 15 per cent in combination with urea were significantly better than their fertilizer controls. N-serve showed deleterious effect on the grain yield at 15 per cent level with urea and this was statistically inferior to lower concentration treatments as well as urea alone.

3. Nitrogen uptake. (i) By grain. Perusal of Table 3 shows that all the treatments of karanjin improved the uptake of nitrogen by rice grain. N-serve though equally effective with ammonium sulphate failed to show any significant uptake with urea except at 10 per cent level. As in the case of dry matter production, it significantly reduced the nitrogen uptake at 15 per cent dosage. Treatments with 10 and 15 per cent karanjin with ammonium sulphate, although not significantly different amongst themselves, were significantly better than 5 per cent level. With urea, there was no significant difference between the treatments with different levels of karanjin. N-serve performed best at 10 per cent level with both the fertilizers.

(ii) By dry matter (grain + straw). It is seen from Table 3 that with karanjin, the nitrogen uptake by total dry matter followed the same trend as the nitrogen uptake by grain, with both the fertilizers. The highest uptake was obtained at 10 per cent level with ammonium sulphate and 15 per cent level with urea. With N-serve, 10 per cent level was again the best with both the fertilizers.

CONCLUSION

The advantageous role of 'N-serve' nitrification inhibitor on growth and yield of rice crop has been observed by many workers[4]. The present studies show that pure karanjin is equally active and must be the factor responsible for the activity albeit milder of the crude extracts of cake and seed of *P. glabra* observed by earlier workers.

The present studies further reveal that the furan ring in karanjin is essential for this activity as dihydrokaranjin, in which the furan ring is reduced, does not posses any inhibitory effect. Other modifications, where the furan ring is kept intact, retain this effect in varying degrees.

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