Karanja *(Pongamia Glabra Vent)* as Source of Nitrification Inhibitors*

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Abstract

The nitrification inhibitory activity of various constituents of Karanja *(Pongamia glabra) have been investigated at the Division of Agricultural Chemicals, Indian Agricultural Research Institute, New Delhi, since* 1966 *and this work is described in the present paper.* Karanja *seed cake, extracts of* Karanja *seed and bark have been reported to possess nitrification inhibitory activity. Also Karanjin, the major furanoflavonoid from the* Karanja *seed is found to be a potent nutrification inhibitor. It equals Nitrapyrin (N-serve) in its performance in retarding nitrification of urea and ammonium sulphate as well as in increasing the yield, N uptake and grain protein of rice in green house pot studies. Structure-activity relationship with Karanjin and chemically altered molecules from Karanjin show that its furan ring is essential for imparting nitrification inhibitory activity.*

The work reported suggests that non-fatty minor constituents of Karanja *seed (as alcohol extract) and Karanjin, a major furanoflavonoid from* Karanja *seed can be advantageously used for retarding nitrification and improving the efficiency of fertiliser nitrogen. These indigenous materials merit their field evaluation as nitrification inhibitors based on their reported promising performance* **in laboratory and** *greenhouse pot experiments.*

Introduction

Nitrogen is vital for increasing crop production. But the efficiency of fertiliser nitrogen is frustrating low especially under tropical agriculture (1, 9, 13). Among the various approaches followed for increasing and conserving fertiliser nitrogen, the use of chemicals that can retard nitrification have been investigated extensively during the past two decades (2, 3, 9, 11, 12). Nitrification inhibitors when added with ammoniacal or ammonium forming fertilisers retard nitrification and thus help in minimising the subsequent losses by denitrification and leaching. These chemicals prove effective in increasing the efficiency of fertiliser nitrogen under situations, where losses due to denitrification and leaching accompanying nitrification are high (12).

It has also been recognised that for nitrification inhibitors to be used under field conditions, they have to be cheap and abundantly available in addition to being effective in retarding nitrification at reasonable rates of application. There is an obvious need to develop cheap and effective nitrification inhibitors from indigenous resources so that their use is economically feasible in different regions of the country. This paper describes the work done at the Division of Agricultural Chemicals, Indian Agricultural Research Institute, New Delhi, for developing nitrification inhibitors from *Karanja* (*Pongamia glabra*) (Table 1). The nitrification inhibitory properties of leaves, seed and bark extracts of *Karanja* are described. Karanjin, the major furanoflavonoid from the *Karanja* seeds has been investigated in details and found to be a potent nitrification inhibitor. As noted earlier, the major portion of the literature comes from the work done at the Division of Agricultural Chemicals, Indian Agricultural Research Institute, New Delhi, initiated under the direction of late Professor K. C. Gulati.

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Non-fatty Minor Constituents of Karanja Cake as Nitrification Inhibitors

Non-edible oil seed cakes particularly *Karanja* and *Neem* (*Azadirachta indica*) have been used as source of nutrients since long. It is believed that in addition to supplying plant nutrients when applied to soil, they may also control soil borne insects. The various constituents isolated from different parts of *Karanja* tree also have medicinal, insecticidal and bactericidal uses. The various constituents of *Karanja* and their uses have been described in details by Parmar *et al.* (7) and here only the nitrification inhibitory activities of these constituents will be dealt. For the manurial value and nitrifiability of various non-edible oil seed cakes including *Karanja*, the reader is also referred to the earlier studies of Plyman and Bal (8), Pal and Rakshit (6), Khan (4) and Yashwant *et al.* (20).

Oil cakes in general and non-edible oil cakes in particular have been known to possess certain non-fatty minor constituents, which impart some physiological activity to them. The presence of several furanoflavonoids in *Karanja* seeds, bark, leaves and flowers has been very well established and summarised (7). The non-edible character of the oil seeds is ascribed mainly due to the presence of these non-fatty minor constituents, which also impart biological and physiological activity to them. Sinha (19) reported that the solvent extraction of the

Table 1—Constituents of Karanja (Pongamia glabra) tested as nitrification inhibitors.

Particular of the constituent	Description of the constituents	References
Karanja seed cake	Ground Karanj seed are defatted by extraction with boiling petroleum ether and the seed cake used for evaluation	Singh (18)
Karanja leaves	The leaves are dried and ground before use	Sahrawat *et al* (17)
Karanja seed extract	The ground seeds are first defatted with petroleum ether and the cake is then extracted with boiling ethanol (95%), solvent removed to obtain the alcohol extract, which is used for testing without further purification	Sahrawat *et al* (17)
Karanja bark extract	Fresh bark of the tree is ground and extracted with 40:60 (v/v) mixture of petroleum ether: acetone, solvent removed to obtain the extract	Sahrawat *et al* (17)
Karanjin, a furanoflavonoid from Karanja seed	Karanjin, a crystalline solid with molecular formula $C_{18}H_{12}O_4$ and chemically 3-methoxy furano-2', 3',7,8-flavone is prepared from karanja seed as described by Sahrawat and Mukerjee (15). Chemically altered compounds from karanjin were also prepared and tested for structure-activity relationship studies	Sahrawat (10); Sahrawat and Mukerjee (15)

Neem seed cake improved nitrificability of the cake, which was attributed to the removal of the bitters.

Singh (18) studied the effects of *Karanja* seed cake and its extractives on nitrification in details and reported that the addition of the crude extractives from *Karanja* seeds to ammonium sulphate resulted in accumulation of ammonium and regulation of nitrate formation in soils. The nitrification inhibitory effect was observed between 25 and 50 days of application in laboratory incubation studies.

Sahrawat *et al.* (17) investigated the nitrification inhibitory activity of the *Karanja* seed and bark extracts and leaves. The alcohol extract of *Karanja* seeds and petroleum ether and acetone extract of the *Karanja* tree bark effectively inhibited nitrification of ammonium sulphate and urea nitrogen in a sandy clay loam. Nitrification of urea was slowed down by the seed and bark extracts upto 45 days. The percentage inhibition of nitrification of urea in soil was 47-55 per cent even after 45 days of application of these extracts (Table 2). The application of leaves, however, had little or no effect on nitrification of ammonium sulphate or urea nitrogen in the soil.

Karanjin, the Major Furanoflavonoid from Karanja Seed as Nitrification Inhibitor

Sahrawat (10) investigated in details, the nitrification inhibitory activity of Karanjin (3-methoxy furano-2', 3', 7, 8-flavone) isolated from the *Karanja* seeds. Karanjin proved to be a very potent inhibitor of nitrification and equals Nitrapyrin (N-serve) in its performance in retarding nitrification in soils treated with ammonium sulphate or urea. The inhibition of nitrification of urea or ammonium sulphate ranged from 43 to 49 per cent after 8

*Table 2—Inhibition of nitrification in a sandy loam soil fertilised with urea (200 ppm N) by karanja seed and bark extracts**

Test inhibitor	Amount applied (per cent of N added)	Per cent inhibition of nitrification after days				
		15	30	45	60	75
Seed extract	20	56	18	47	10	0
	30	67	56	50	10	<1
Bark extract	20	42	11	50	3	0
	30	53	33	55	4	6

*Soil samples in duplicate were incubated under aerobic conditions (1/3 W.H.C. moisture) at 30 ± 2°C.

$$\text{Per cent inhibition of nitrification} = \frac{(NO_2^- + NO_3^-)\text{—N in no inhibitory treated soil} - (NO_2^- + NO_3^-)\text{—N in inhibitor treated soil}}{(NO_2^- + NO_3^-)\text{—N in no inhibitor treated soil}} \times 100$$

Source: Adopted from Sahrawat *et al* (17).

*Table 3—Inhibition of nitrification in a sandy loam soil fertilised with ammonium sulphate (A.S.) or urea (200 ppm N) by Karanjin as compared to Nitrapyrin (N-serve)**

Treatment		Per cent inhibition of nitrification after weeks							
Fertiliser	Inhibitor	1	2	3	4	5	6	7	8
A.S.	Karanjin	64	70	71	65	54	51	51	43
A.S.	Nitrapyrin	64	78	78	77	65	53	54	44
Urea	Karanjin	72	71	71	65	53	58	51	43
Urea	Nitrapyrin	78	77	78	78	65	58	52	49

*The inhibitors were applied at the rate of 5 per cent of the fertiliser nitrogen added.

$$\text{Per cent inhibition of nitrification} = \frac{(NO^-_2+NO^-_3)\text{—N in no inhibitor treated soil}—(NO^-_2+NO^-_3)\text{—N in inhibitor treated soil}}{(NO^-_2+NO^-_3)\text{—N in no inhibitor treated soil}} \times 100$$

Source: Adopted from Sahrawat (12)

weeks of incubation when Karanjin and Nitrapyrin were added at the rate of 5 per cent of the fertiliser nitrogen applied (Table 3).

Like Nitrapyrin, Karanjin specifically inhibits the first step of nitrification, viz., conversion of NH_4^+ to NO_2^- mediated by *Nitrosomonas* bacteria without affecting the subsequent oxidation of NO^-_2 to NO^-_3 by *Nitrobacter*.

Nitrobacter

Structure-activity relationship studies with Karanjin and chemically altered molecules from Karanjin showed that its furan ring is essential for imparting the nitrification inhibitory effect. Dihydro Karanjin not having a furan ring (prepared by hydrogenation of Karanjin) did not show appreciable nitrification inhibitory effects as compared to karanjin, karanj ketone, and karanjonol (all having a furan ring in the molecule) which all showed nitrification inhibitory activity to varying degrees (15).

Results on the inhibition of nitrification of urea N in Table 4 clearly bring out this point. The hypothesis that furan moiety in a molecule imparts nitrification inhibitory properties to varying degrees was further supported by later studies where various compounds having furan ring were tested and found to retard nitrification of urea and ammonium sulphate nitrogen (5, 16).

Effects of Karanjin on Growth, N Uptake and Grain Protein of Rice

The data obtained in a greenhouse pot experiment showed that

*Table 4—Effects of karanjin, karanj ketone, karanjonol and dihydrokaranjin on nitrification in a sandy loam soil fertilised with Urea (200 ppm N)**

Inhibitor	Furan ring in the molecule present or absent	Per cent inhibition of nitrification after 15 days of incubation
Karanjin	Present	47
Karanj ketone	Present	44
Karanjonol	Present	28
Dihydrokaranjin	Absent	0

*All the test nitrification inhibitors were added at the rate of 5 per cent of the fertilisr N applied.

Source: Adopted from Sahrawat and Mukerjee (15).

Table 5—Effects of inhibiting nitrification of ammonium sulphate and urea fertilisers by karanjin and nitrapyrin on grain and straw yields (g/pot) of rice grown in pots.

Treatment	Ammonium sulphate		Urea	
	Grain	Grain+Straw	Grain	Grain+Straw
Fertiliser alone (150 kg N/ha)	27.3	64.3	29.3	65.7
+ 5 per cent Karanjin	36.0	78.0	40.7	79.4
+ 5 ,, Nitrapyrin	36.0	76.3	36.0	78.0
+10 ,, Karanjin	42.0	79.3	41.0	75.7
+10 ,, Nitrapyrin	41.0	81.0	40.0	73.7
+15 ,, Karanjin	40.3	74.3	41.0	82.3
+15 ,, Nitrapyrin	36.0	74.7	23.3	48.3
C.D. (0.05)	3.66	7.76	4.88	6.69

Source: Sahrawat and Mukerjee (15).

the addition of Karanjin or Nitrapyrin to ammonium sulphate or urea significantly improved the grain and total dry matter yield of rice (variety 'Bala') (Table 5). The various levels of nitrification inhibitors gave non-significant difference in yield of rice except in case where Nitrapyrin was applied at 15 per cent, level with urea that significantly lowered the yield (Table 5). Nitrapyrin has deleterious effect at the highest rate with urea on yield of rice which proved inferior to the lower rates of Nitrapyrin as well as to urea alone treatments.

As with grain and dry matter yield, the nitrogen uptake of rice (Table 6) and grain protein content (Table 7) were increased by inhibiting nitrification of ammonium sulphate and urea. Nitrapyrin gave the best results with both the fertilisers at 10 per cent level and its 15 per cent rate with urea proved significantly inferior as observed for grain and dry matter yield.

Karanjin at the 10 and 15 per cent levels and Nitrapyrin at the 10 per cent level significantly increased grain protein. Rice protein levels were highest at the 15 per cent Karanjin level (Table 7). The various treatments of Karanjin with ammonium sulphate and urea fertilisers increased the grain yield by 31-54 per cent; grain + straw yield by 21-25 per cent: total N uptake by 36-68 per cent and grain protein of rice by 2-14 per cent in these studies (Sahrawat 12).

The conservation of ammonium nitrogen in the soil due to application of the nitrification inhibitors is further indicated by the results of analysis of the soil samples after harvest of the rice crop (Table 8). The soils with fertiliser plus inhibitor treatments revealed significantly higher amounts of ammoniacal nitrogen than those treated with the fertilisers alone. The nitrate contents of soil samples receiving the inhibitors were found to be significantly lower than the untreated fertiliser treatments (Table 8). The lowest amount of nitrate was observed with 15 per cent Karanjin and Nitrapyrin treatments.

Conclusions

The work described in the paper clearly brings out the potentiality as inhibitors of nitrification of the

Table 6—Effects of inhibiting nitrification of ammonium sulphate and urea on nitrogen uptake (mg N/pot) of rice grown in pots.

Treatment	Ammonium sulphate		Urea	
	Grain	Grain+Straw	Grain	Grain+Straw
Fertiliser alone (150 kg N/ha)	339.1	503.4	359.7	513.0
+ 5 per cent 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
C.D. (0.05)	65.28	50.64	113.09	67.22

Source: Sahrawat and Mukerjee (15).

Table 7—Effects of inhibiting nitrification of ammonium sulphate and urea by Karanjin and Nitrapyrin on rice grain protein.

Treatment	With ammonium sulphate	With Urea
	*Protein content, per cent	
Fertiliser alone (150 kg N/ha)	7.76ab	7.67a
+ 5 per cent Karanjin	7.99bc	7.81a
+ 5 ,, Nitrapyrin	7.41a	7.52a
+10 ,, Karanjin	8.28c	8.34b
+10 ,, Nitrapyrin	8.22c	8.60b
+15 ,, Karanjin	8.84d	8.54b
+15 ,, Nitrapyrin	8.05bc	7.56a

*Values followed by a common letter within a column are not significantly different at the 5 per cent level based on Duncan's New Multiple Range Test.

Source: Sahrawat and Mukerjee (14).

Table 8—Effects in Karanjin and Nitrapyrin on ammoniacal and nitrate nitrogen content in soil after harvest of rice crop.

Treatmetn	With ammonium sulphate		With urea	
	NH^+_4—N	NO^-_3—N	NH^+_4—N	NO^-_3—N
No inhibitor	6.60c	7.90a	6.50c	7.45a
5 per cent Karanjin	9.45b	7.00b	9.35b	6.90b
5 per cent Nitrapyrin	9.55ab	6.20c	9.40b	6.70b
10 ,, Karanjin	9.50b	7.00b	9.66b	5.90c
10 ,, Nitrapyrin	9.87ab	6.12c	9.80ab	6.00c
15 ,, Karanjin	10.80a	6.32c	10.30a	6.00c
15 ,, Nitrapyrin	10.00ab	6.12c	10.25a	5.75c

*Means followed by a common letter in each column are not significantly different at 5 per cent level based on DMRT.

Source: Sahrawat and Mukerjee (14).

various constituents of *Karanja* (*Pongamia glabra*). Among these: the alcohol extract of the seed cake, which contain the biologically active non-fatty minor constituents of the seed holds promise as an indigenous and cheap material. The bark extract also possesses nitrification inhibitory activity worth exploitation.

Karanjin, a furanoflavonoid from *Karanja* seed has proved to be a very promising nitrification inhibitor both in laboratory and greenhouse pot experiments with rice crop. It increased the yield, nitrogen uptake and grain protein of rice in the greenhouse studies and matched in performance to the well known nitrification inhibitor Nitrapyrin (N-serve). Based on the reported promising performance in laboratory and greenhouse pot studies, these indigenous and cheap material merit their field evaluations as nitrification inhibitor for enhancing the efficiency of fertiliser nitrogen under situations where due to poor control of water, losses by denitrification and leaching are high.

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