

Unexploited botanical nitrification inhibitors prepared from Karanja plant

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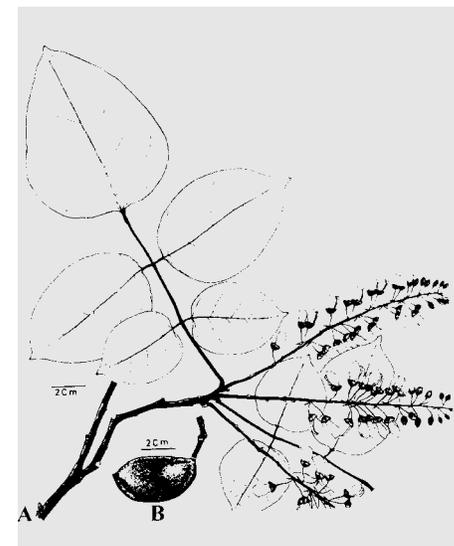
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Abstract

Karanjin, a furanoflavonoid (3-methoxy furano-2', 3', 7, 8-flavone, $C_{18}H_{12}O_4$), is the non-fatty component of oil extracted from the seeds of Karanja tree (*Pongamia pinnata* Pierre syn. *P. glabra* Vent.). Karanjin content in seeds is highly variable yielding up to 1.25% (w/w). It is reported as early as in 1925, has been found to be a very potent nitrification inhibitor (NI), sometimes comparable or even more potent than a few commercialized NIs in soils but unfortunately has not been used widely in agricultural fields or commercialized as a nitrification inhibitor. It has also been found to minimize nitrous oxide (N_2O) emissions from soil *in vitro*. The use of karanjin as a nitrification inhibitor is yet to be made in field and hence, more research on karanjin in relation to cost effective extraction and purification process, etc. should be given an emphasis. In the present communication an attempt has been made to give an up-to-date account of unexploited botanical nitrification inhibitors prepared from karanja plant.

Keywords: Agriculture, Fertilizer, Nitrogen, Nitrification inhibitors, Karanjin, *Pongamia pinnata*, Karanja.

IPC code; Int. cl.⁸ — C05C, C09K 17/00



A-Karanja branch bearing fruit and flowers, B-Fruit

Introduction

Nitrogen (N) is the most yield-limiting nutrient in crop production. It is required in highest quantity in soil and is easily lost from the soil via denitrification, volatilization, leaching and run off. Also, being a costly plant nutrient, nitrogen demands the greatest attention for its management¹. Research on the development of nitrification inhibitors (NIs) is being carried out for the last few decades worldwide to tackle the important issue of preventing N losses from applied N fertilizers, increasing crop yield and reducing environmental pollution. Addition of a nitrification inhibitor to soil with ammonium (NH_4^+) containing fertilizers or urea retards nitrification and minimizes leaching of nitrogen as nitrate

(NO_3^-) and gaseous losses of nitrogen as NO , N_2O and N_2 via denitrification². Furthermore, nitrification inhibitors also suppress both methane emissions and methane oxidation^{3,4}. Various chemically synthesized chemicals have been introduced in the past as NIs and their efficacy in N conservation and increasing crop yield has been demonstrated in incubation, pot culture and field studies^{2, 5-8}. But, large-scale use of NIs in agricultural fields has never been done, due to lack of research, knowledge in farmers, poor agricultural extension and absence of regulatory controls on its use in agriculture. Extra expenditure on NIs has added to their unpopularity in India and other developing countries. Botanical NIs have been prepared and tested in India

for quite sometime especially those prepared from neem (cakes and oil based products, etc.), but they also have failed to reach farmers' fields by and large except few localized uses^{9,10}. A report chronicles the use of Sweet Wormwood (*Artemisia annua* Linn.) and Spearmint (*Mentha spicata* Linn.) oil with urea as NIs for N conservation¹¹.

Karanjin, extracted and prepared from seeds, bark and leaves of karanja tree, though found to be a potent NI several decades back^{12, 13}, has been neglected and experimental research on its use as a NI has almost ceased in India except a few endeavors in very recent times^{14, 15}. The present communication will look into the

evolution of karanjin as a NI, available scientific literature on its success as an NI and also the reasons for its failure to penetrate Indian *vis-a-vis* world agricultural package of practices.

Karanja, *Pongamia pinnata* Pierre (syn. *P. glabra* Vent.) also known as Pongam oil tree or Indian Beech is known by various local names in India like *Karanj* or *Karanja* (Hindi, Bengali, Marathi and Gujarati), *Gaanuga* or *Pungu* (Telugu), *Ponga* or *Pongam* (Tamil), *Honge* (Kannad), *Pungu* or *Punnu* (Malayalam), *Koranjo* (Oriya), *Sukhchein*, *Karanjpaphri* (Punjab and Kumaun) and *Karchaw* (Assam). It is a medium sized, glabrous, drought and salinity resistant, frost hardy, semi-deciduous nitrogen fixing leguminous tree¹⁶. It has a short bole and spreading crown, up to 18 m high or even more and 1.5 m girth. Its bark is greyish green or brown, smooth or covered with tubercles and leaves are imparipinnate and leaflets 5-7 ovate or elliptic. Flowers are lilac or white, tinged with pink or violet and are fragrant. Pods containing seeds are compressed, woody, indehiscent, elliptic to obliquely oblong, with a short covered beak. They bear usually one seed, which are elliptical or reniform. The tree starts bearing at 4-7 years and seed yield is anywhere between 9-90 kg per tree. Seed collection is basically a rural activity and is unorganized. Small agencies and forest contractors organize collection and transport. The removal of kernel from pod is done manually. The kernels are tough white in colour, covered with thin reddish brittle skin. In dry pods, the shell and kernel are 4.5 and 95.5 %, respectively. The composition of a sample of air-dried kernels in per cent is as

follows: moisture 19.0, oil 27.5, protein 17.4, starch 6.6, crude fibre 7.3 and ash 2.3 (Ref. 17).

Karanja is considered to be a native to Western Ghats and is primarily found along banks of streams and rivers or near sea coast in beach and tidal forests. It is found almost all over India up to an altitude of 1,200 m and distributed further eastwards, chiefly in the littoral regions of South-Eastern Asia and Australia, East Fiji. It is well adapted to all soil types and climatic requirements and grows in dry places also. It is a shade bearer and is considered to be a good tree for planting in pastures, as grass grows well in its shade. The tree is suitable for afforestation especially in watershed areas and in drier part of the country. Andhra Pradesh, Tamil Nadu and Karnataka provide the bulk of seeds of this tree. A large number of karanja trees have been planted on roadside both on highways and also in urban areas during last two decades¹⁷.

Nitrification inhibition in soil and its importance

Nitrification inhibitors prevent or inhibit nitrification i.e., conversion of NH_4^+ to NO_3^- by selectively inhibiting microbial enzymes responsible for conversion of NH_4^+ to NO_2^- , thereby slowing down the formation of NO_3^- from NH_4^+ (Refs. 2, 18). Thus, nitrification inhibitors are those chemical compounds that inhibit the first step of nitrification, resulting in a preponderance of ammonium (NH_4^+) over nitrate (NO_3^-) in soil. This results in higher persistence of applied N in the soil and root zone since NH_4^+ gets fixed in soil cation exchange sites thereby preventing its immediate loss,

influencing N uptake by plants, their N nutrition and yield. On the contrary, without the presence of a NI, easily formed NO_3^- would have been lost very quickly from soil due to its high solubility in water and lesser fixation to less prevalent anion exchange sites in soil¹⁹. Although some of the conserved NH_4^+ is lost via ammonia (NH_3) volatilization, which is an environmental concern since NH_3 leads to changes in atmospheric chemistry^{20,21}, but nevertheless, NIs saves appreciable amount of applied and residual soil NH_4^+ , which would otherwise be lost via quick nitrification to NO_3^- (Refs. 5, 19, 22, 23). Nitrification inhibitors also affect nitrogen transformations other than nitrification in soils such as ammonium fixation and release, mineralization and immobilization, nitrous oxide production and ammonia volatilization, which affects N persistence in the soil and its subsequent availability to plants²⁴.

Various chemically synthesized nitrification inhibitors have been tried in agriculture for a long time, e.g. nitrapyrin or N-serve [2-chloro-6-(trichloromethyl) pyridine], dicyandiamide or DCD or cyanoguanidine [$\text{HN}=\text{C}(\text{NH}_2)-\text{NH}-\text{CN}$], potassium azide (KN_3), 2,4-diamino-6-trichloromethyl-strizine, AM (2-amino-4-chloro-6-methylpyrimidine), ST (2-sulphanilamido thiazole), DCS (N-2, 5-dichloro phenyl succinamic acid), thiosulphates, thiourea, ASU (1-amindino-2-thio urea, a thiourea derivative), MBT (2-mercapto-benzothiazole), diethyl-di-thio-carbamate, sodium chlorate, encapsulated calcium carbide (a source of acetylene), carbon disulphide (CS_2) to name the majority^{5, 25-35}. Although several of the said NIs have been commercialized, most of these chemical NIs have seldom

Table 1 : Some plant products tested as nitrification inhibitors

| Class of materials / compounds | Reference |
|---|------------|
| Karanja seed cake and its isolates | 13, 36, 37 |
| Karanjin, a furano-flavonoid from Karanja seed | 36, 38, 39 |
| <i>Azadirachta indica</i> A. Juss. cake and its isolates | 6, 40, 41 |
| <i>Citrullus colocynthis</i> (Linn.) Schrad. cake | 42 |
| <i>Madhuca indica</i> J. E. Gmel. syn. <i>Bassia latifolia</i> Roxb. (Mahua) cake | 43, 44 |
| Vegetable tannin, waste tea | 45-47 |
| Nimin, patented Neem oil based triterpene | 6, 7, 48 |
| Mint essential oil | 49 |

been used by farmers in developing and underdeveloped countries and very occasionally in developed countries due to extra cost incurred, marginal economic return and lack of government push and absence of any usage legislation.

Plant products as nitrification inhibitors

Apart from chemically synthesized nitrification inhibitors, many plant products especially non-edible oil seeds and their constituents have been evaluated as sources of chemicals for retarding nitrification. Some important works on the use of botanical materials as nitrification inhibitor are summarized in Table 1. Among the non-edible oil seeds, neem (*Azadirachta indica* A. Juss.) and karanja cakes and their constituents have been traditionally used in admixtures with manures and have since been extensively evaluated for retarding nitrification in the soil^{6, 12, 38, 39, 40, 50, 51}.

Chemical composition of Karanja seeds

The yield of kernels per tree is reported to be between 8 to 24 kg and oil content 27-39%. Fresh extracted non-edible oil is yellowish orange to brown,

darkens during storage, and has disagreeable odour and bitter taste⁵²; the oil is known as pongam oil in trade¹⁷. Solvent extraction yields better quality of oil. The physico-chemical properties of the oil are: refractive index 1.434-1.479 at 40°C; specific gravity 0.925-0.940 at 30°C; iodine value 80-96; acid value >20; saponification value 185-195 and unsaponifiable value 2.6-3.0. The seeds also contain mucilage (13.5%) and traces of essential oil. In addition, a complex amino acid glabrin is also present. Four furanoflavones namely karanjin, pongapin, kanjone, pongaglabrone and diketone pongamol have been isolated from the Indian seeds while in Australian seeds only pongapin is present. Karanjin and pongamol, associated with non-glyceride portion of oil, are the major flavones with bitter taste. The seed oil is known as pongam oil in trade¹⁷.

Karanjin: the furano-flavonoid

Karanjin (Fig. 1a), the principal furano-flavonoid constituent of karanja was the first crystalline compound reported as early as 1925^(Ref. 12). It was isolated from the karanja seed oil and roots and its structure, constitution and synthesis were thoroughly investigated by

several workers. It was followed by the discovery of pongamol¹². Karanjin has recently been isolated from the petrol extract of *Lonchocarpus latifolius* H. B. & K. roots including 9 other flavonoids, viz. karanjachromene, lanceolatin B, pongachromene, pongaglabrone and pongapin⁵³. Various furano and chromene derivatives of karanja possess angular orientation of the furan or the chromene ring. Further, most of these are derivatives of resorcinol in so far as ring A is concerned. The methoxylated derivatives as observed in the chromeno chalkones, viz. glabrachromene I and II, and pongachalkone, have also been discovered¹². Various other derivatives like

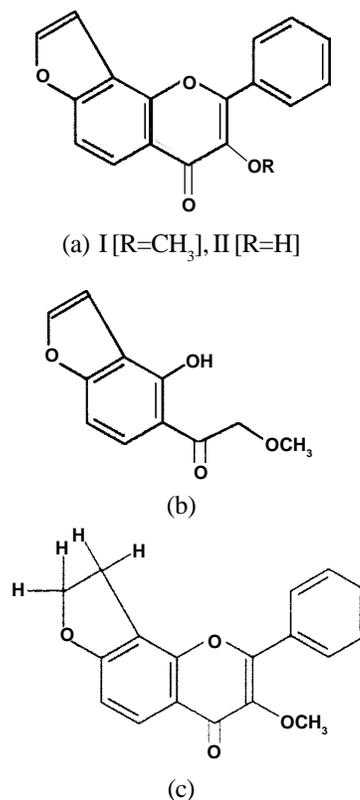


Fig. 1 a-c: Chemical structures (a) Karanjin-I and Karanjonol -II (b) Karanj ketone, and (c) Dihydrokaranjin

karanj ketone (4-hydroxy-5- ω -methoxy acetyl coumarone) (Fig. 1b), dihydrokaranjin (3-methoxy dihydrofurano-2', 3', 7, 8 flavone) (Fig. 1c) and karanjonol (3-hydroxy furano-2', 3', 7, 8-flavone) have been prepared from karanjin^{5,4}.

Karanjin, chemically 3-methoxy furano-2', 3', 7, 8-flavone, mol formula C₁₈H₁₂O₄, a non-fatty component of oil, slowly separates out from the oil on standing⁵⁵, at a faster rate at low temperature (<4°C). Yields up to 1.25% of karanjin and 0.85% of pongamol have been reported¹². So, older samples of oil have lesser karanjin percentage. Karanj content in seeds is highly variable, depending on place of origin and season of collection of seeds. Even from de-oiled oilcakes karanjin can be extracted and its amount was found to be 0.37 and 0.4% on fresh and dry de-oiled oilcake weight basis⁵⁶. Crude karanjin obtained on standing can later on be purified and crystallized into needle like crystals⁵⁷. In another study⁵⁸, residual karanjin in raw and variously processed cake was quantified using high performance liquid chromatography (HPLC) where the raw expeller karanja cake was found to contain about 0.19% of karanjin. Though a non-polar solvent, soxhlet extraction of expeller pressed cake with petroleum ether drastically reduced karanjin content (0.01%). Soaking of cake for 24 h in 1% NaOH (w/w) solution reduces karanjin content with little further benefit by increasing alkali level. Milder alkali like lime and fertilizer grade urea reduces the karanjin levels marginally. Similar was the case with mineral acids such as HCl and glacial acetic acid. It was, therefore, concluded that solvent extraction of seeds

would be the best method of detoxification as well as for more recovery of oil and karanjin.

Karanjin is responsible for karanja oil's action against skin diseases. Karanj possesses insecticidal and antibacterial properties and is also highly toxic to fish. A 2% solution of karanjin is marketed in the name of Derisom (2% EC) as an insecticide, acaricide and fungicide by an Indian company, proclaimed by the company as the first karanjin biopesticide in world market. Studies conducted at National Botanical Research Institute (NBRI), Lucknow, have shown that methanolic extract of karanja oil had maximum antifeedant and growth reduction activity on polyphagous crop pest, *Spodoptera litura* due to presence of high concentration of karanjin and pongamol while methanolic extract of bark showed good antifeedant and growth regulatory activity against the same pest. An effective oil based formulation having azadirachtin-enriched fraction of neem oil and karanjin enriched fraction of karanja oil is developed by the same institute for control of *S. litura*. In another study, karanja oil was found to be most toxic followed by neem and mahua while combination of neem and karanja (1:1) was less toxic than individual oils alone on spider mites (*Tetranychus urticae*)⁵⁹. Karanj in dilutions up to 10⁻⁵ was found to suppress the growth of *Mycobacterium tuberculosis* H₃₇ Rv^(Ref. 17). Karanj solution (in acetone) of 500, 1000 and 5000 μ g/ml did not have any inhibitory action on *Azotobacter chroococcum* and *Azospirillum lipoferum* in a disk diffusion assay test⁶⁰. Among other uses, methylated esters of karanja oil has been

used with blends of diesel and tested for its fuel properties and engine performance and was found to have reduced exhaust emissions (NO_x, CO, smoke) and increased torque and brake power⁶¹.

Estimation of karanjin in plant biomass and oil

Estimation of karanjin in plant biomass (e.g. seed, bark, leaf, oil cake, etc.) can be done by extracting, precipitating and crystallizing and then determining the mass of crystallized karanjin since its concentration in these samples is appreciable whereas it has to be estimated by a sophisticated analytical instrument like HPLC in samples containing trace amounts. Karanj can be separated along with other flavones by Wakosil-II %C 18 RS column, using CH₃CN (0.05 M) and NaH₂PO₄ (12/88, v/v) as the mobile phase (flow rate 1.0 ml/min at 40°C), the varying proportions of which changes the run time from 8 to 55 minutes. Detection was done by a UV detector at 325 nm^(Ref. 62).

Karanjin has also been analyzed by reverse phase high performance liquid chromatography (RP-HPLC). This method required no sample preparation of active ingredients. The analysis used methanol, water and acetic acid in the ratio of 85:13.5:1.5 as mobile phase at a flow rate of 0.5 ml/min on a Kromasil 100 C18 column, ex Tracer (250 \times 46mm, particle size 5 microns) and dual wavelength detection at 350 and 300 nm, 2-ethylhexyl-p-methoxy cinnamate (Parsol MCX) was used as the internal standard. The analysis was free from substrate interference. The detection limits for karanjin were found to be 0.1 μ g/ml^(Ref. 63).

Research on karanjin as a

Table 2 : Different products and by-products of Karanja proposed as nitrification inhibitors^{50, 64}

| Constituents | Description/preparation of the products |
|----------------------|---|
| Karanja seed cake | The ground seeds are defatted by extraction with boiling petroleum ether and the seed cake (residue) is used for evaluation |
| Karanja leaves | The leaves are dried and ground before use |
| Karanja seed extract | The grounded 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 |
| Karanja bark extract | The fresh bark of the tree is ground and extracted with 40:60 (V/V) mixture of petroleum and ether acetone, solvent removed to obtain the extract |
| Karanjin | Karanjin a crystalline solid with molecular formula C ₁₈ H ₁₂ O ₄ and chemically 3-methoxy furano 2', 3', 7, 8 flavone is obtained from karanja seed. |

nitrification inhibitor

Development and application of karanjin and allied products from karanja plant in agriculture has been done mainly in India^{13, 14, 50, 51, 39, 64-66}. Early literature reported that lipid associates of karanja oil extracted from seeds, seed cakes and bark by ethanol and other solvents such as acetone or petroleum possess nitrification inhibitory property^{13, 50, 51}. Table 2 summarizes different products and by-products of the tree karanja used as nitrification inhibitors. Sahrawat *et al* evaluated the nitrification inhibitory activity of the seeds, bark and leaves of karanja, in a sandy clay loam soil⁵⁰. The defatted seeds were extracted with hot ethanol and the extract was compared with the fresh bark extract (extracted by a mixture of petroleum ether and acetone in the ratio of 40: 60) and ground leaves to evaluate their effects on nitrification in soil fertilized with urea and ammonium sulfate. The results showed that the alcohol extract of the seeds had maximum effect on the conservation of ammonium,

followed by the bark extract while the leaves had little effect. None of the materials tested had any effect on the conversion of nitrite to nitrate. The alcohol extract of the seeds was effective in retarding nitrification for 60 days when added at the rate of 20% of the fertilizer N rate. Sahrawat and Mukerjee⁶⁷ also found karanjin as a potent inhibitor of nitrification in soil when it compared well with the patented nitrification inhibitor nitrapyrin in retarding the nitrification of soil fertilized with urea or ammonium sulfate. The nitrification inhibitory activity of karanjin proved useful in improving the yield and quality of rice in greenhouse pot culture studies. Unfortunately, till date, no field study has been conducted on karanjin. This could be due to lack of research interest and tediousness in the extraction and purification of large quantity karanjin for field application.

Further, where suitable alteration of the karanjin molecule: karanj ketone and karanjonol were prepared from karanjin and tested for their effects on

nitrification, it has been observed that the furan ring present in the molecule is the crucial structural factor for nitrification inhibitory activity. All the compounds with the exception of dihydro karanjin where furan ring was absent, exhibited nitrification retarding ability to varying degrees⁶⁶. Like nitrapyrin, karanjin specifically inhibits the first step or nitrification, viz. conversion of NH₄⁺ to NO₂⁻ mediated by *Nitrosomonas* bacteria without affecting the subsequent oxidation of NO₂⁻ to NO₃⁻ by *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 did not show appreciable nitrification inhibitory effects as compared to karanjin, karanj ketone and karanjonol all of which showed nitrification inhibitory activity to varying degrees⁶⁷. Thus, the furan ring imparts nitrification inhibitory activity was further confirmed by later studies in which several compounds based on furfuraldehyde including furfuraldehyde and furfuryl alcohol showed varying degrees of ability to retard nitrification in soils fertilized with urea^{36, 64}. Results showed that furfural and furfuryl alcohol were effective in retarding nitrification for 30 days when added at a rate of 10% of urea N. Among the several furano compounds evaluated for retarding nitrification, 5-nitro-furfural oxime and furfural oxime were found to be very effective inhibitors of nitrification of urea N in soil. Sahrawat (1978) reported an inhibition of nitrification of urea or ammonium sulphate in the range of 43-49% after 8 weeks of incubation when karanjin and

Table 3 : A summary of available literature on nitrification inhibition by Karanjin and its derivatives

| Product | Dose used (% of N) | Fertilizer | Soil type | Period (days) | Nitrification inhibition (%) | Reference |
|----------------------|--------------------|-------------------|-----------------|---------------|------------------------------|-----------|
| Karanja seed extract | 20 | Urea | Sandy clay loam | 60 | 10 | 50 |
| -do- | 30 | -do- | -do- | -do- | 10 | -do- |
| Karanja seed extract | 20 | -do- | -do- | -do- | 3 | -do- |
| -do- | 30 | -do- | -do- | -do- | 4 | -do- |
| Karanjin | 5 | Urea | Sandy loam | 15 | 47 | 70 |
| Karanj ketone | -do- | -do- | -do- | -do- | 44 | -do- |
| Karanjonol | -do- | -do- | -do- | -do- | 28 | -do- |
| Dihydrokaranjin | -do- | -do- | -do- | -do- | 0 | -do- |
| Karanjin | 5 | -do- | -do- | 56 | 43 | 68 |
| -do- | 5 | Ammonium sulphate | -do- | -do- | 43 | -do- |
| Karanjin | 5 | Urea | Sandy clay loam | 49 | 27 | 36 |
| Karanjin | 5 | -do- | -do- | 60 | 31 | 13 |
| | 10 | -do- | -do- | -do- | 43 | -do- |
| Karanja seed extract | 30 | -do- | -do- | -do- | 5 | -do- |
| Karanjin | 5 | -do- | Sandy loam | 30 | 62 | 14 |
| -do- | 10 | -do- | -do- | -do- | 71 | -do- |
| -do- | 15 | -do- | -do- | -do- | 75 | -do- |
| -do- | 20 | -do- | -do- | -do- | 67 | -do- |
| -do- | 25 | -do- | -do- | -do- | 75 | -do- |
| Karanjin | 20 | -do- | Sandy loam | 42 | 45-54 | 15 |
| -do- | -do- | -do- | Clay | -do- | 4-50 | -do- |
| -do- | -do- | -do- | Sandy | -do- | 43 | -do- |
| -do- | -do- | -do- | Clay loam | -do- | 43 | -do- |
| Karanjin | 5 | -do- | Sandy loam | 35 | 50 | 65 |
| -do- | 10 | -do- | -do- | -do- | 67 | -do- |
| -do- | 15 | -do- | -do- | -do- | 71 | -do- |
| -do- | 20 | -do- | -do- | -do- | 63 | -do- |
| -do- | 25 | -do- | -do- | -do- | 63 | -do- |

the neem extracts at the rate 30% of the N rate was comparable to that of karanjin at 5 and 10% concentration. The extracts of neem and karanjin seeds were effective in retarding nitrification up to 45 days while karanjin was effective up to 60 days. There were some differences in the patterns of inhibitions of nitrification by karanjin and neem seed extracts and based on this, the author suggested that the mixture of extracts of karanja and neem seeds would be a better material for sustained retardation of nitrification in soil than the sole use of either material.

After a long gap, in recent times, karanjin has been tested for nitrification inhibition where application of karanjin resulted in more nitrification inhibition (62-75%) than DCD (9-42%), during a 30 days trial in a Typic Ustochrept alluvial soil¹⁴. In another study, nitrification inhibition by karanjin (applied @20% of applied N) remained appreciable, ranging from 9-76% for seven different soils of India approximately for a period of 6 weeks¹⁵. Available research results on nitrification inhibition by karanjin in soil have been summarized in Table 3.

Regulation of greenhouse gas emissions from soils by karanjin and some other botanical NIs

As a nitrification inhibitor, karanjin is expected to mitigate nitrous oxide (N₂O) emissions from soil by slowing down conversion of NH₄⁺ to NO₂⁻, but was never tested for this role until 2001, when an incubation experiment was conducted to study N₂O emission from a Typic Ustochrept, alluvial soil, fertilized with urea and urea combined with different levels of karanjin and DCD. Karanjin and DCD were incorporated @

nitrapyrin were added at the rate of 5% of the fertilizer nitrogen applied⁶⁸. Comparative evaluation of karanjin and three patented nitrification inhibitors, nitrapyrin, AM (2-amino-4-chloro-6-methyl pyrimidine) and DCD (dicyandiamide) for retardation of nitrification of urea in a sandy clay loam

soil showed that the effectiveness of the compounds tested decreased in the order: nitrapyrin>karanjin>AM>dicyandiamide⁶⁹.

Sahrawat (1982) compared alcohol extracts of karanja and neem seeds to retard nitrification of urea in a sandy clay loam in an incubation experiment¹³. The treatment of urea with

5, 10, 15, 20 and 25% of applied urea-N (100 mg/kg soil), to the soil adjusted at field capacity moisture content. Karanjin appreciably reduced the mean N_2O flux from urea-treated soils and also total N_2O -N emissions by 92-96% and was more potent than DCD (60-71%)^(Ref. 14).

Karanjin was found to have a regulatory effect on CH_4 consumption by soil *in vitro*. Several studies had suggested that application of NH_4^+ -N decreased the capacity of soils to oxidize CH_4 ^(Refs. 71-73), since NH_4^+ leads to an increase in nitrifier population relative to methanotrophs, reducing overall CH_4 oxidation, as nitrifiers oxidize CH_4 less efficiently than methanotrophs⁷⁰. In a Typic Ustochrept (alluvial Inceptisol) soil, collected from a field under rice-wheat rotation, fertilized with urea (100 mg N/kg soil) and urea combined with different doses of karanjin (at 5, 10, 15, 20 and 25% of applied N), incubated at 25°C, CH_4 consumption rate was found to be higher in control (no fertilizer-N) than N treatments. Mean CH_4 consumption rate, as well as total CH_4 consumption was lower after the addition of karanjin due to slower nitrification and higher conservation of NH_4^+ . Addition of urea led to 17% reduction of total CH_4 consumption while urea combined with karanjin had 50-64% reduction, respectively⁶⁵.

Other botanical nitrification inhibitors, especially neem based, have also been tested for their role in influencing greenhouse gas emissions from soils and crops. Neem cake coated urea has been found to reduce total N_2O emission by 10.5 and 11.2%^(Ref. 6, 8) from rice and 4.9 and 21.2% from wheat^{7, 8}.

Another patented neem based NI, Nimin, marketed by Godrej Agrovet Ltd. had reportedly reduced N_2O emissions by 4.9 and 30% from rice and wheat, respectively^{6, 7}. A neem oil coated urea had reportedly reduced 21 and 15.2% total N_2O emission from rice and wheat, respectively⁸. The neem cake coated and neem oil coated ureas have mitigated CH_4 emissions by 14.9 and 7.8%, respectively, from rice⁸.

Conclusion

Though there is difficulty in extraction and crystallization and its production *en masse* in the pure form due to its very low content in karanja oil. Karanjin can definitely be considered worth experimenting with as a NI. But, research on product development and its use in agriculture assumes a high place of importance since it is a known nitrification inhibitor of high efficiency, the only challenge remains to produce it cheaply and quickly in large amounts, its popularization, marketing and large scale use in agriculture based on its success under field conditions. As a welcome relief, some private chemical and agrochemical companies have started producing karanjin based formulations albeit for pesticidal use, but in the process karanjin has come into the fore. These pesticides might also have some nitrification inhibitory properties due to presence of karanjin, which have to be tested for a possibility of its use as a NI. As a financial incentive, industries producing karanjin based formulations could incorporate the product in clean development mechanism (CDM) projects, since it is capable of reducing N_2O emissions from crop fields, to earn credits

known as Certified Emission Reductions (CER), which it can trade with other industries.

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