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Stability of azadirachtin: A tetranortriterpenoid from Neem tree

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Abstract
Azadirachtin-A is the most active secondary metabolite possessing various pesticidal properties among a number of liminoids isolated from Neem (*Azadirachta indica*) seeds. It has a complex molecular structure and possesses reactive functional groups susceptible to oxidative degradation. Owing to the presence of acid sensitive groups like tertiary hydroxyls and a dihydrofuran ring it is highly unstable under acidic conditions, while the presence of four ester groups makes it equally unstable under alkaline conditions. Various physical parameters such as, temperature, moisture and light effect differently on most vulnerable sites of the molecule which, lead to its degradation under these conditions. In addition, interaction of solvent molecules with active functionalities also results in chemical changes in the molecule. The stability of Azadirachtin-A also varies in different storage environments due to one or several factors described above. An understanding of its degradation mechanism and effect of various factors influencing stability of Azadirachtin will be of great help for development of stable formulations and effective utilization of Neem products. The chemistry of active sites of Azadirachtin, possible molecular changes under mild conditions, degradation kinetics and mechanisms has been reviewed in this publication.

Keywords: Neem, azadirachtin stability, limonoids

Introduction
Neem tree (*Azadirachtin indica* a. Juss) has been known for centuries for its medicinal properties, while its use for the control of insect was discovered only in the first half of the last century. More than 200 compounds were reported from various parts of the Neem tree [1], but the bitter principles of Neem, mainly limonoids were responsible for the insect control activity. Of all these components, Azadirachtins which are abundantly present in Neem seed kernel are potential agents for insect control. Several commercial products have been developed from the crude and semi-refined extracts of Neem seed kernel, Neem oil and most of them were standardized based on active components such as Azadirachtins [2]. Azadirachtin-A and Azadirachtin-B are considered as the main active components for regulatory clearances in many countries for the Neem products used for insect control in non-food and food crops. The stability of Azadirachtin in Neem seed extracts, its formulated products, spray mix and in plant tissues, decides its effectiveness for crop protection against various insects. Azadirachtin has a great potential as a safe insect control agent but it is a challenging task for the formulation chemists to develop a successful commercial product based on inherent unstable nature of Azadirachtin molecule. The success lies in controlling Azadirachtin degradation in various forms during storage and in field conditions.

The Azadirachtin molecule is reported to degrade rapidly in Neem seed kernel, in extracts, in pure forms and as well as in combination with other ingredients during storage. The extent of degradation varies in different storage conditions due to one or several factors. The molecule has eight condensed ring, three carbocyclic and five heterocyclic, the later of which contain oxygen as hetero atom which include one tetra- substituted oxirane, two five-membered, one tetrahydrofuran and one six membered pyran rings. Other oxygen functionalities include an enol ether, acetal, hemiacetal, secondary and tertiary hydroxyl groups and a variety of carboxylic esters and a tiglate linkage. The chemical reactivity of various functions of azadirachtin molecule was extensively studied [3] and some of its degraded products were characterized [4], which created better understanding on aza stability. There have been continuous efforts all over the world to increase the stability of azadirachtin. In this publication we have reviewed the literature on chemical activity of various functions of Azadirachtin

molecule which likely affect its stability, effect of the factors such as light, humidity, pH and temperature on its stability and its reported stability in natural source of neem seed kernel, extracts, various solvents and formulations etc.

Chemistry of active sites in azadirachtin

Azadirachtin is one of the most highly oxidized limonoids known. It is a complex molecule which boasts a plethora of oxygen functionality, comprising an enol ether, acetal, hemiacetal, and tetra-substituted oxirane as well as a variety of carboxylic esters. Additionally, both secondary and tertiary hydroxyl groups and a tetrahydrofuran ether are present. The structure of the molecule is given below-

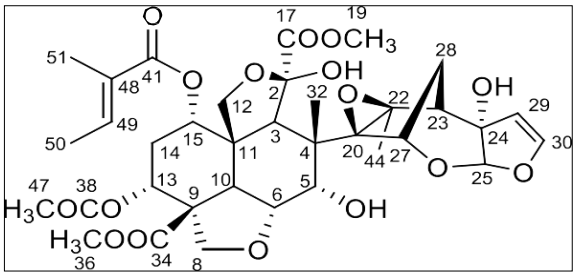

Fig 1: Azadirachtin A

An inspection of the molecular structure reveals 16 stereogenic centres, seven of which are quaternary. The instability of Azadirachtin was attributed partly due to the presence of the tiglate and enol ether residues in the molecule. The reason for Azadirachtin degradation may be predicted by understanding chemical reactivity of different functions under diverse chemical and physical conditions. The reactivity of some of the functional groups has been outlined below:

Hydrolysis reactions

The azadirachtin A molecule is reported to undergo hydrolysis at pH 2, 4, 6 and 8 and some of its degradation products were identified by HPLC-MS and HPLC-MS-MS analysis [4], as detailed below:

Product 1: The molar mass of the degradation product (P1) of Azadirachtin A at pH 2 was 724 g mol^{-1}. This mass is higher than that of azadirachtin-A (720 g mol^{-1}) by 4 amu. The structure of this product can be attributed on the basis of the hydrolysis of one of the two ester groups in azadirachtin-A at positions 2 and 9, and by addition of one molecule of water to the double bond at position 48-49 as shown in Figure 2.

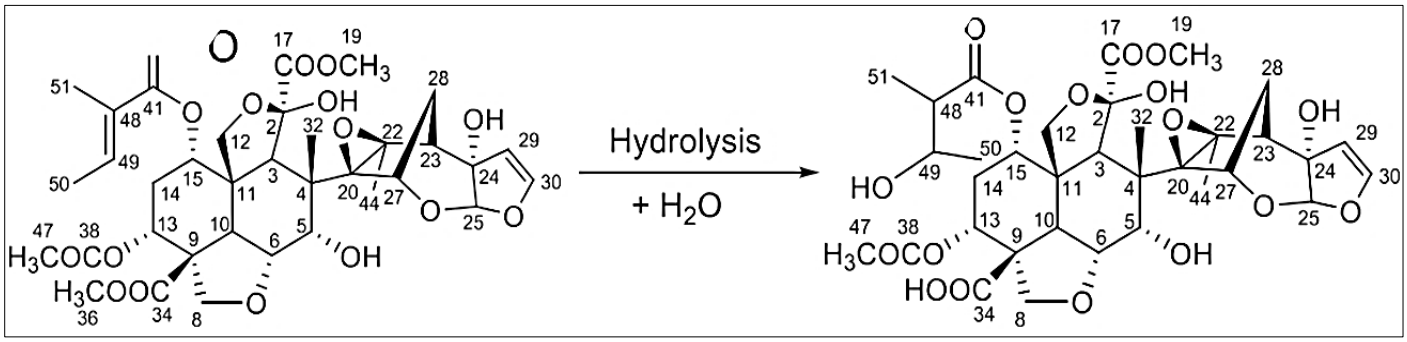

Fig 2: Reaction pathway for degradation product P1

Product 2: The molar mass of one more degradation product (P2) of azadirachtin A at pH 2 was 688 g mol^{-1}, equivalent to a loss of a mass of 32 g mol^{-1} from the starting product azadirachtin-A. Under these pH conditions the transformation can be assumed by hydrolysis and loss of one water molecule from position 24–25, as shown in the reaction path given below in Figure 3.

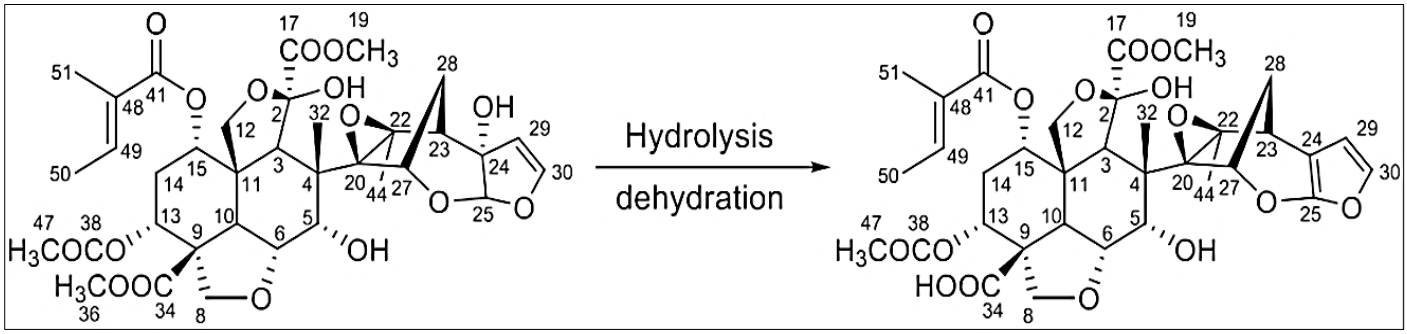

Fig 3: Reaction pathway for degradation product P2

Product 3: The molar mass of this degradation product (P3) of azadirachtin A was 724 g mol^{-1} and P3 is a positional isomer of product P1. The degradation pathway is given below in Figure 4.

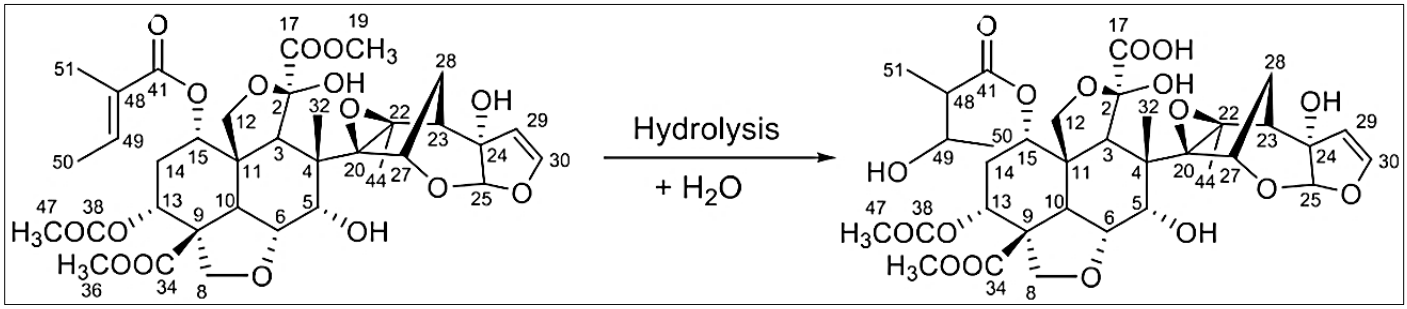

Fig 4: Reaction pathway for degradation product P3

Product 4: The degradation product (P4) is identified to be a positional isomer of product P2. The hydrolysis reaction involves the ester function at C-2. A water molecule was eliminated between the hydroxyl group on carbon 5 and the hydrogen on carbon 6. The reaction path way is given below in Figure 5.

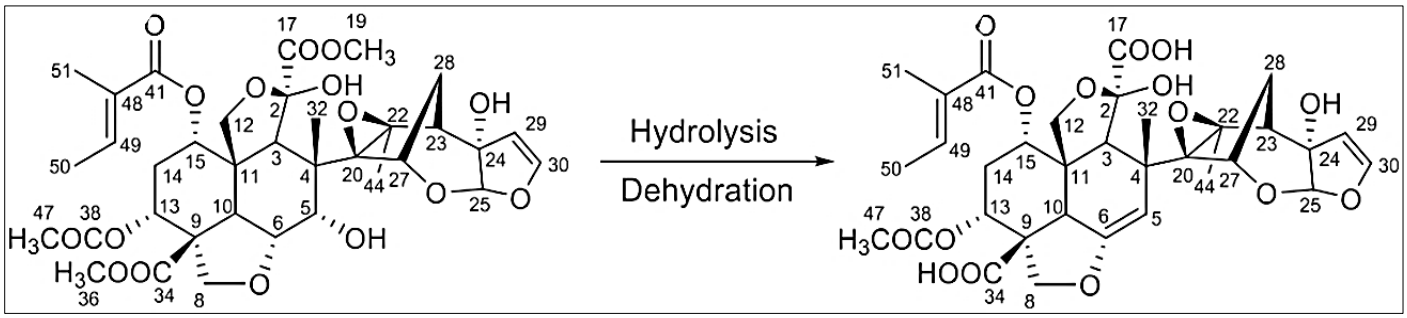

Fig 5: Reaction pathway for degradation product P4

Product 5: The molar mass of the degradation product (P5) of azadirachtin A was 679 g mol^{-1}, equivalent to loss of 41 Da from the starting product azadirachtin-A. This result can be interpreted as the substitution of the acetate group by hydroxyl *via* deacetylation as shown in Figure 6.

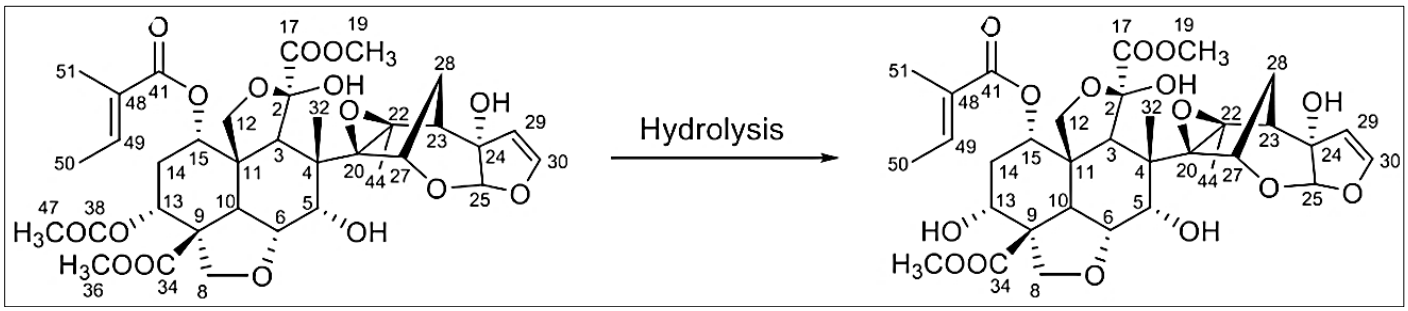

Fig 6: Reaction pathway for degradation product P4

Product 6: The molar mass of the degradation product (P6) of azadirachtin A is 706 g mol^{-1}, corresponding to loss of 14 g mol^{-1} from the starting product. This product is obtained at pH 4, 6 and 8 and its kinetics of appearance is accelerated in alkaline medium. The mass spectra analysis indicates that one of its ester functions is hydrolyzed. The reaction path way is given below in Figure 7.

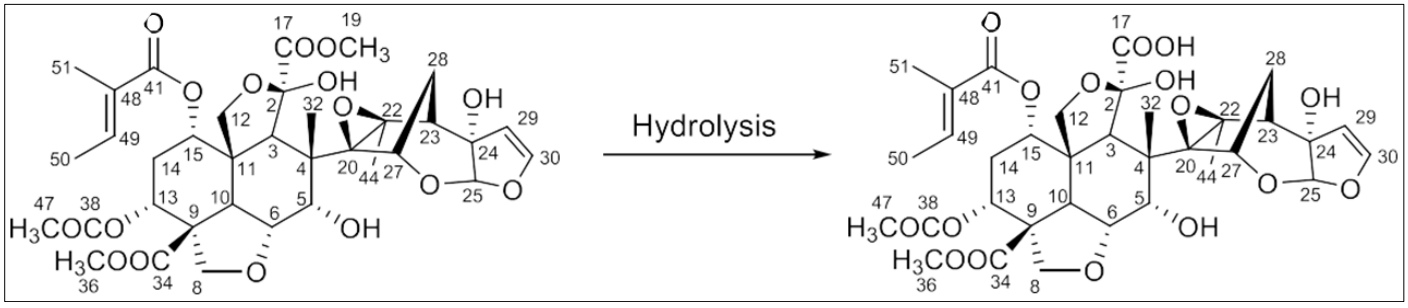

Fig 7: Reaction pathway for degradation product P6

Product 7: The molar mass of the degradation product (P7) of Azadirachtin A is 692 g mol^{-1}, which is corresponding to loss of 28 g mol^{-1} from the starting product. This can be interpreted in terms of hydrolysis of two ester functions in the molecule, results consistent with the chemical structure shown in Figure 8.

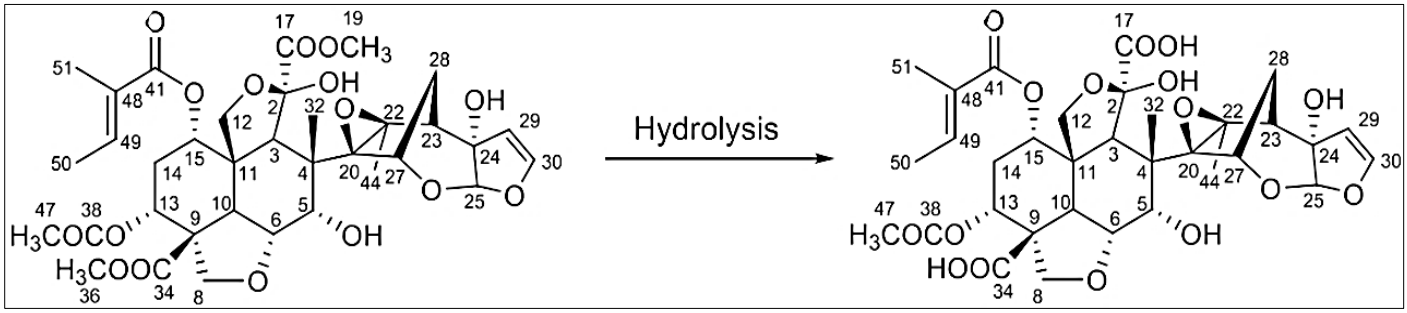

Fig 8: Reaction pathway for degradation product P7

Product 8: The degradation product P8 results from transformation of product P7 by the loss of a water molecule from its precursor. The mass spectra of [M-H] daughter ions reveals the loss of 196 Da, yielding a peak at m/z 477. This result proves that dehydration occurs between the hydroxyl group on carbon 5 and the hydrogen on carbon 6. The proposed chemical structure of this degradation product is shown in Figure 9.

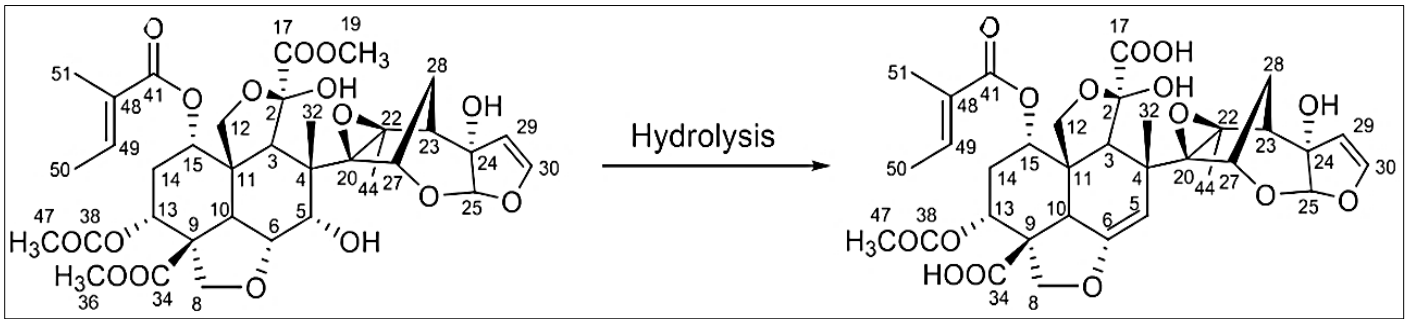

Fig 9: Reaction pathway for degradation product P8

Hydroxyl group reactivity

Two prominent hydrogen bonds were observed in azadirachtin-A molecule one of which being strong between the C(2)-OH and oxirane oxygen and another significantly weaker one between the C(24)-OH and C(5)-OH in which the former group functioning as hydrogen bond donor. These intramolecular hydrogen bonds partly determine the activity of hydroxyl groups. The hydroxyl group reactivity was reported to be in the pattern of C(2)-OH>C(24)-OH>>C(5)-OH owing to the easy formation of a C-2 acetate on treatment of azadirachtin-A with acetic anhydride [5a-d] and formation of 2, 24-dicarbomethoxyazadirachtin [5b] by heating with dimethyl pyrocarbonate. Harsh conditions were needed for silylation of the secondary C(5)-OH group providing an early indication of the unreactivity of this functional group [3].

Reaction of C-29-30 enol ether

S.V. Ley *et al*. [6a-b] has demonstrated that reaction of azadirachtin-A with acetic acid gave a mixture of anomeric acetates. Interestingly, these anomeric acetates were re-converted to azadirachtin-A by pyrolytic *syn*-elimination of acetic acid. Other carboxylic acids such as formic and propionic acids, etc. failed to give similar products.

Saponification reactions

The saponification of Azadirachtin with methanol under basic conditions resulted in lower yield of 3-desacetylazadirachtin [5b, c]. Both acetyl and tigloyl residues were found to be less reactive towards hydrolysis. The difficulty for hydrolysis of the tiglate ester was attributed to steric hindrance and selective hydrolysis was achieved after conversion of tiglate to a highly reactive C-1 pyruvate by osmium catalyzed oxidation.

Oxidation reactions

Azadirachtin failed to oxidize under normal conditions [5c], and varied activity of three hydroxyl groups was observed towards oxidation. Activated DMSO reagents and ruthenium-based oxidants failed to oxidize, however, pyridinium dichromate was successful [7]. The C (7)-OH group was highly resistant to oxidation due to shielding of this function by the right-hand side of the molecule with a network of hydrogen bonds.

Skeletal rearrangements

In addition to inherent chemical reactivity of constituent functional groups of azadirachtin-A with external agents, synergistic interactions among these groups within the molecule were observed to trigger unusual rearrangement reactions. The azadirachtin to azadirachtin in rearrangement, which presumably arises by C(2)-OH intramolecular oxirane ring-opening in a SN2 fashion with accompanying inversion at C-14, is the best understood and most easily recognized example of this behavior [8]. Conversion of Azadirachtin to 3-acetyl-1-tigloylazadirachtinin was reported by Jarvis et. al. [9], when Azadirachtin is heated in methanol at 90 ^{O}C. Another unusual rearrangement was reported which involve formation of oxetane ring formation by 7-*endo-tet* cyclization and induces C-13-C-14 oxirane ring opening (Baldwin, 1976) [10]. The resulting C(14)OH group then causes trans acetalization at C-11 liberating an angular C-19 hydroxy methylene ‘arm’ which lactonizes at the C-29 ester.

Influence of environmental factors such as light, temperature, humidity, pH etc., on stability of Azadirachtin

Effect of light on azadirachtin stability

The exposure of Azadirachtin with UV light in presence of oxygen or nitrogen atmosphere resulted in conversion of (E)-2-methylbuty-2-enoate ester group into its Z- isomer, Johnson, *et al*. (1994) [11a]. Dureja *et al*., (2000) 11a and Johnson, *et al*., 2000), has also reported that exposure of azadirachtin-A to UV light (254 nm), as a solid thin film on glass surface gives a single photoproduct by conversion of (E)-2-methylbut-2-enoate ester group of azadirachtin-A into (Z)-2-methylbut-2-enoate ester (Figure 10) [11]. Half-life of azadirachtin-A as thin film under UV light was found to be only 48 min.

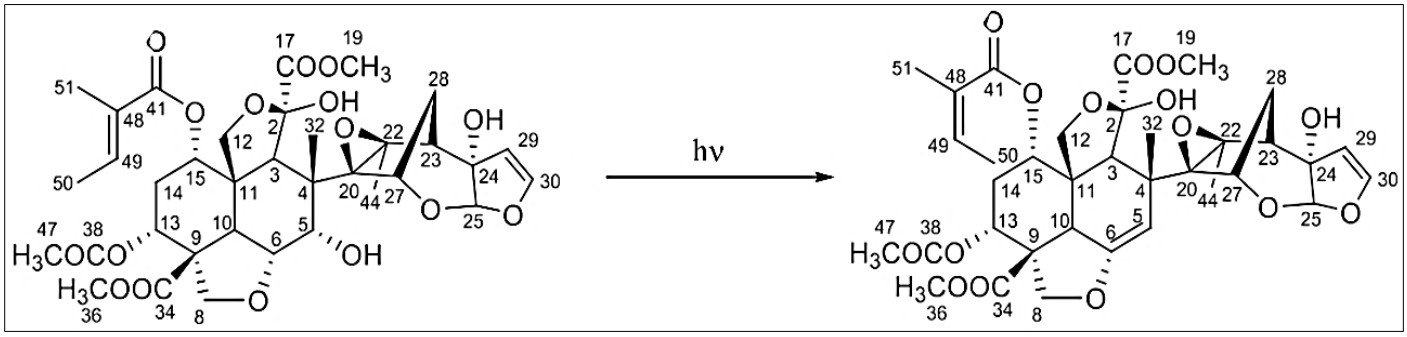

Fig 10: Structure of Azadirachtin A and its photoproduct

The UV-labile nature of azadirachtin was studied by Klocke *et al*. [12]. Rapid degradation of azadirachtin was observed when its ethanolic solution was exposed to UV light of 200-280 nm at room temperature. Under these conditions the compound appeared to be degraded in a first order reaction with T1/2 as 25 hrs. The same had been observed in 13-deacetyl-aza and 29, 30-dihydro-aza and suggested that the α, β–unsaturated carboxyl (in tigloyl form) could be the major site of chemical degradation. In this support, 29, 30, 48, 49-tetrahydroazadirechtin, which does not have tigloyl moiety, found to be not UV-labile. UV-absorbing substances such as *p*-amino benzoic acid, lecithin were claimed to prevent degradation of azadirachtin-A by sun light [13].

The half-life of azadirachtin was 48 min and 3.98 days, as a thin film under UV light and sunlight, respectively. 8-Hydroxyquinoline and *tert*-butylhydroquinone have been found effective in controlling degradation of azadirachtin under both sunlight and UV light with half-life of 44.42 and 35.90 days under sunlight, and 55.80 and 48.50 hr under UV light, respectively [14]. Photo oxidation of azadirachtin-A by UV light in presence of oxygen proceeded slower than that of nimbin and salannin. All photoproducts showed some biological activity against *Spodoptera littoralis*, *Locusta migratoria*, and *Schistocerca gregaria* [15] Azadirachtin and two of its derivatives 3-deacetylazadirachtin, and 22,23-dihydroazadirachtin photodegraded rapidly by only 90 hrs of exposure to UV radiation, but one or more of their degradation products was as biologically active as the parent molecule [16].

A number of stabilizers have been reported which protect Azadirachtin molecule from UV light degradation of Azadirachtin. A list of some of the most effective UV stabilizers has been given below in Table 1.

Table 1: Recommended UV stabilizers for azadirachtin A.

S. No.	UV Stabilizer	Reference
1	*p*-amino benzoic acid; lecithin	13
2	8-Hydroxyquinoline; tert-butylhydroquinone	14
3	2, 4-dihydroxybenzophenone (Uvinul M-400)	13a
4	Phenyl salicylate	17
5	Non-ionic surfactants Emulsol-N-33	18
6	Natural absorber-Aloin (in 1:1 mol ratio)	19

Azadirachtins and related compounds were found to be very sensitive to sunlight, degrading rapidly, with half-lives of the order of 11.3 h for azadirachtin A and 5.5 h for azadirachtin B and few minutes for the other limonoids compounds e.g., salannin, nimbin, deacetylnimbin, and deacetylsalannin. Caboni, *et al*., (2006) [20] and Stokes, *et al*. (1982) [21] have have demonstrated that sun light causes about 50% degradation of azadirachtin in pure form following 7 days exposure. The photodegradation of azadirachtin in presence of epicuticular waxes extracted from the olives showed rapid degradation with half-life as 0.8 days [22].

Azadirachtin was unstable under normal conditions of storage of neem seeds. Azadirachtin content reduced to about 68% of the original level in a period of 4 months in the dark and to 55% in daylight [23].

Effect of temperature on azadirachtin stability

The effect of temperature on degradation kinetics was studied at the same pH and noticed that the rate of disappearance of azadirachtin-A increased with temperature independently of pH [4]. Azadirachtin A was quantitatively converted to 3-acetyl-1-tigloylazadirachtinin at 90 ^{O}C in methanol. The half-life of azadirachtin-A in methanol at 50 ^{O}C and 90 ^{O}C was found to be 6.96 days and 11.7 hour respectively [9].

The degradation rates of Azadirachtin in four solvents were in the order of ethanol>methanol>DMF>acetonitrile, with 45.3%, 38.7%, 27.5, and 22.3% degradation after 14 days of storage of the methanol extracts at 54±10 C temperature. Pure azadirachtin dissolved in the same solvents decomposed 62.6%, 41.9%, 28.0%, and 11.1%, respectively under identical conditions. After the storage at 0±1 °C for 14 days, azadirachtin in all treatments showed almost no decomposition and only slight degradation at room temperature [24].

Degradation rates of azadirachtin A powder in methanol, methanol + water (9:1, vol./vol.) and methanol + neem oil (9:1, vol./vol.) at 54±1°C in 7 days were 29.65%, 44.99% and 12.76% respectively. Degradation of azadirachtin A powder stored at 54±1°C for 14 day was 90.15%. Degradation rates of azadirachtin A in neem seed methanol extracts were 82.35% and 94.79% after 7 day and 14 day of storage at 54±1°C temperature [25].

Nearly 96-99% of azadirachtin A in emulsifiable concentrations (aza-A content = 617.93-1149.65 ppm) degraded during the heat stability test at 54±1°C for 14 days with half- lives ranging between 1.84 and 4.53 days [26]. Formulation of azadirachtin A on attapulgite, kaolinite, fuller's earth, hydrated calcium silicate, and fly ash caused degradation of azadirachtin A by 70-95%, as compared to 56% in neem oil, during a 14 days storage at 54°C, in the lab. The degradation was reduced by 26-60% on different carriers by employing either anthraquinone or epichlorohydrin as stabilizer. Pyrogallol and hydroquinone enhanced the degradation [27]. Azadirachtin A and the sum of azadirachtins decreased by 18 and 15%, respectively during 2-year storage of NeemAzal-T/S [28] Azadirachtin was reasonably stable (95-99%) in a variety of solvents such as chloroform, acetone, ethyl acetate, acetonitrile, methanol and ethanol at room temperature for 7 days and to ultrasound for 30 minutes [29]. A pesticide composition containing 3.2% azadirachtin in an aromatic petroleum distillate and 10% 1, 2-epoxyoctane showed 2.8% azadirachtin content after 28-day storage at 50° temperature [30].

Effect of pH on azadirachtin stability

Azadirachtin A was hydrolysed rapidly in pond water at pH 10, and the DT50 was only about 2 hr. At pH 4, the DT50 values for the pure and formulated azadirachtin A were 19.2 and 38.3 days, respectively, indicating that the chemical is relatively stable in acidic medium. The stability was diminished at pH 7 and the corresponding DT50 values were 12.9 and 30.5 day for pure and formulated Azadirachtin A [31].

The disappearance of Azadirachtin A in four natural waters of pH 6.2, 7.3, 8.0 and 8.1 followed simple pseudo-first-order kinetics with half-life values of 256, 43.9, 10.2 and 14.2 hour, respectively. The rate of disappearance was more rapid at basic than at acidic pH.32

Azadirachtin was reported to be unstable in mildly alkaline and strongly acidic solutions and most stable in mildly acidic solutions between pH 4 and 6, at room temperature. Its half-life in distilled water (pH 6.7) at 50oC and 90 ^{O}C was 14.9 hour and 18 minute respectively. In phosphate buffer (pH 7), its half-life was 9.9 hour and 8.9 minute at 50 ^{O}C and 90 ^{O}C temperature respectively [9].

Effect of humidity/water on azadirachtin stability

Azadirachtin decomposed completely in for less than 7 days at (54 ± 1) °C temperature in water [33]. The half-life (DT50) value of azadirachtin in stream water was about 35 h regardless of the dosage rate applied. The duration of Azadirachtin persistence in terrestrial and aquatic matrixes of a forest ecosystem in Ontario, Canada, varied from 3 to 6 days in terrestrial matrixes, whereas it ranged from 8 to 13 days in water, and 2 to 3 days in sediment of aquatic matrixes [34]. The half-life (t1/2) of azadirachtin A in water (pH = 4, 30 ºC) and soil was 9.8 and 1.8 days, respectively. In addition to the hydrolytic degradation in open waters, the photochemical degradation was t1/2 = 10 hour [28].

The dissipation (DT50) of azadirachtin-A, B and total residue in bottom sediments or pore water of a typical forest pond of northern Ontario was 25, 45, and 30 days respectively and 90% dissipation (DT90) was 55, 93 and 66 days respectively [35]. The degradation of Azadirachtin in solid preparations containing Azadirachtin was reduced if the preparation contains about ≤5% volatile polar solvents and <1% water [36].

Azadirachtin degradation in plant tissues

The half-life of azadirachtin on leaf surface under simulated field conditions was 0.56 day, whereas its half-life increased to 1.65 days when aza A was mixed with BHA and Neem oil in 1:1:1 ratio [37]. The disappearance of Azadirachtin from foliage on to balsam fir and oak seedlings in a lab chamber was rapid, and the DT50 values ranged from 17 to 22 hour [38]. The half-life (DT50) and rate-constant (C) values for the pure Azadirachtin A on maple foliage with and without lecithin were 8.77 d and 0.079, and 6.54 d and 0.106 [39]. The half-life (t1/2) of azadirachtin A was 1 day when it was used to protect apples28. Azadirachtin was reported field stable for less than three months [40].

The dissipation half-life (DT50) of azadirachtin A was 16.9 days in shoots and 29.0 days in needles of young spruce trees. The loss of Aza-A in the spruce foliage was due to physical processes such as volatilization, photolysis and metabolic activities [41]. On application of a nutrient solution containing azadirachtin A (10 µg/mL) on young spruce tree, the Azadirachtin A was taken up by roots and translocated in shoots and needles. A peak concentration of 5.16 + 0.73 µg/g and 2.56 + 0.31 µg/g was reached after 8 day and 15 day of post-treatment in shoots and needles and the residue gradually decreased to 3.61 + 0.48 µg/g and 2.41 + 0.29 µg/g respectively after 20th day of post-treatment [42].

The adsorption and persistence of azadirachtin in the target matrix is influenced to a considerable degree by the formulation recipes and the additives present in the spray mixtures. The dissipation half-lives in foliar residues [dislodgeable residues (DR), penetrated residues (PR) and total residues (TR)], onto potted spruce seedlings in a laboratory spray chamber ranged from 19.5 to 38.9 hour and varied according to the residue type and formulation used (WP or EC formulation). The DT50 values of the DR were consistently lower (range, 19.5 to 31.9 h) than those of the PR (range, 30.5 to 38.9 h) due to preferential loss of the surface residues. The DT50 values for the DR and TR were low in the foliage sprayed with the WP spray mix compared to EC spray mixes [43].

Azadirachtin degradation in soil

The half-life of Azadirachtin A in autoclaved and non-autoclaved nursery soils, at 21+2 ºC temperature and 30+2% moisture was 35.6 days and 25.8 days, respectively [42]. The disappearance of Azadirachtin-A residues (half-life) in potted nursery soil of aspen seedlings was reported to be 25.77 days, Sundaram, *et al*. (1995) [44], while Tross, et. al. (1997) [28] have reported that the half-life azadirachtin A is as low as 1.8 day in soil. The breakdown of azadirachtin A in the soil amended with neem leaves and animal manure was higher with higher level of neem leaves (100 g neem leaves/ Kg soil) and gave shorter half-life in the range of 12.19 to 22.29 days. The half-life was longer in the range of 28.63 to 42.26 days with lower levels of neem leaves (50 g neem leaves/ Kg soil) [45].

Azadirachtin stability in various solvents

The effect of various solvents on stability of azadirachtin in extracts and its formulations was widely studied. Substantial drop of Azadirachtin was noticed during storage of its extracts in protic solvents which contain acidic or basic functional groups specifically water, acids and bases [46]. Higher stability of azadirachtin was reported in alcoholic and other aprotic solvents which are neutral in nature (Table 2).

Table 2: List of solvents recommended for stable Azadirachtin compositions.

Aprotic solvents		
Acetone	Propylene carbonate	Sulfolane
Acetonitrile	Ethylene carbonate	methyl-t-butyl ether
2-butanone	3-methyl-2-butanone	Xylenes
chloroform trichloroethane	dibutyl ether	Ethyl acetate
Amyl acetate	Chlorobenzene	Cyclohexanone
Dimethylsulfoxide	Propyl acetate	Methylene chloride
Dimethylformamide	chloroform trichloroethane	
Dimethylacetamide	Ethylene chloride	
Diethyl carbonate	Benzaldehyde	
Alcohols		
Ethanol	Methanol	propanol
isopropanol	butanol	2-butanol
t-butanol	benzyl alcohol	

Azadirachtin 10,000 ppm EC formulation Econeem Plus containing a mixture of solvent n- butanol, butyl acetate and neem oil was found stable with 93.6% Azadirachtin retention after 21 days of storage at 54 + 1 deg. C temperature (in-house study-PJ Margo, Pvt. Ltd., Bangalore, India).

Microbial degradation of azadirachtin

Stark et. al. [47] have reported that microbial activity is responsible for faster degradation of azadirachtin in non-autoclaved soils as compared to autoclaved soils. The DT50 values for azadirachtin A in autoclaved and non-autoclaved soil at 15 and 25°C temperature were found to be 91.2 d, 31.5 d and 43.9 d, 19.8 days, respectively. The half-life of Azadirachtin-A in a gallon gum-based minimal medium (M-medium) containing mycorrhizal fungus *G. intraradices* was 44.4 days. The half-lives for technical and formulated Azadirachtin-A ranged from 13.2 to 46.2 days in the systems containing vermiculite, vermiculite and soil mixture, mycorrhizal fungus and leeks [48].

Conclusion

The molecular architecture of azadirachtin molecule is rather complex possessing number of rings and diverse functional groups with varied chemical reactivity. Azadirachtin is highly susceptible to mild variation in chemical conditions and undergoes molecular changes such as hydrolysis, oxidation, acetylation, saponification etc. and rearrangements such as Azadirachtin to Azadirachtin in. Both the tigloyl and enol ether moieties were found to be more susceptible to these changes, leading to degradation of the molecule. Moisture has also been identified to cause rapid degradation of azadirachtin. Chemical nature of the media containing azadirachtin also influences its stability. Azadirachtin was found stable in aprotic and neutral solvents as compared to protic solvents. Azadirachtin was unstable in mildly alkaline and strongly acidic solutions and most stable in mildly acidic solutions between pH 4 and 6. The stability of Azadirachtin in different forms such as powder/liquid was found to be influenced by various physical parameters such as humidity, light and temperature. Individual effect of these parameters has been widely studied but very little is known about combined effect of these factors, which is practically applicable when azadirachtin is used in field conditions to control agricultural insects/ pests. The degradation of azadirachtin in autoclave and non-autoclave soil has been studied by very few authors, but its fate in various types of soil microbes and soils from different climatic conditions is not known and needs to be further studied in details. Many efforts have been made to reduce its degradation in various liquid and solid formulations and its stability has been achieved in solid formulations free from organic solvent residues and less than 1% water, but still there is a lot of gap to overcome its stability problem in liquid formulations and needs further attention. This review provides complete analysis of the active sites of Azadirachtin molecule and effect of various environmental factors leading its degradation, which will facilitate the chemists to develop stable formulations for effective utilization of the molecule.

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