

## Meliacin contents during different reproductive phenophases in *Azadirachta indica* A. Juss

Pesticidal properties of neem-based formulations are due to the presence of bioactive limonoids such as azadirachtins, nimbin, salanin, etc.<sup>1-5</sup>. Although ripe fruits are the source of these bioactive limonoids, there is little information about their presence in flowers and green fruits, during different reproductive phenophases. To understand the pattern of meliacin formation during different reproductive phases, 10 candidate plus trees (CPTs) at different locations around Jodhpur district were selected by the procedure given by Tewari *et al.*<sup>6</sup>. All the CPTs were registered with NBPGR, New Delhi with specific IC numbers (JD-1, IC 268481; JD-2, IC 268482; JD-3, IC 268483; JD-4, IC 268484; JD-5, IC 268485; JD-6, IC 268486; JD-8, IC 268488; JD-13, IC 268393; JD-14, IC 268394; JD-15, IC 268395).

The basic objective of this paper was to generate information on presence of meliacins, during different reproductive phases, therefore, reproductive phenological events were recorded in selected CPTs from initiation of inflorescence formation to fruit maturation (Table 1). Observations were taken precisely at 4–6 day intervals. If a given reproductive phenological event was initiated in five CPTs (50%), it was considered started. If a reproductive phenological event lasted a week or less, it was considered very brief activity; if it lasted more than a week but less than two weeks, it was considered brief activity and the reproductive phenological event, which continued more than two weeks, was considered as extended activity<sup>7,8</sup>. For

analysing meliacins, i.e. azadirachtin-A (AA), azadirachtin-B (AB), nimbin (N), salanin (S), 18 influences were marked in each CPT (six each in lower, middle and upper portion of crown). At each reproductive phenological event, test material was collected from these marked inflorescences. AA, AB, N and S were analysed using standard HPLC procedure with known operating conditions<sup>9</sup>. For computing meliacins, data were averaged across all selected CPTs considering them a single population.

Not a minute fraction of any meliacin was detected in any CPT during extended activity of inflorescence and flower bud formation (event I), and brief activity of flower bud opening and early flowering (event II). During the extended activity of blossoming (event III), AA and AB were detected in few CPTs. On an average, across CPTs, this value was 9 ppm for AA and 2.3 ppm for AB (Figure 1). The average value of N was 18 ppm and that of S was 12.7 ppm in this event. Up to formation of green fruits (event IV), AA and AB were not detected in any CPTs except JD-13. Thus, in the event IV, average value across all the CPTs was 2.5 and 2.0 ppm respectively, while at this stage the average value of N and S was 41 and 158 ppm respectively (Table 2).

Though the formation of AA and AB was very poor till the increment in size of green fruits (event VI, a brief activity), the formation of N and S exhibited accelerated pace from the early stage of fruit formation (event V, a brief activity). On an average across all CPTs, at event

VII, the value of AA and AB was 70.0 and 16.6 ppm respectively, while it was 301.0 and 474.0 ppm for N and S respectively. After event VII, when fruits became yellow and pulpy (event VIII, a brief activity), content of each meliacin exhibited rapid increase and in fully mature fruit till fruit fall, an extended activity (event X), the average content of AA and AB was recorded to be 1201 and 402 ppm respectively, while at this stage, content of N and S was 1161 and 1207 ppm respectively.

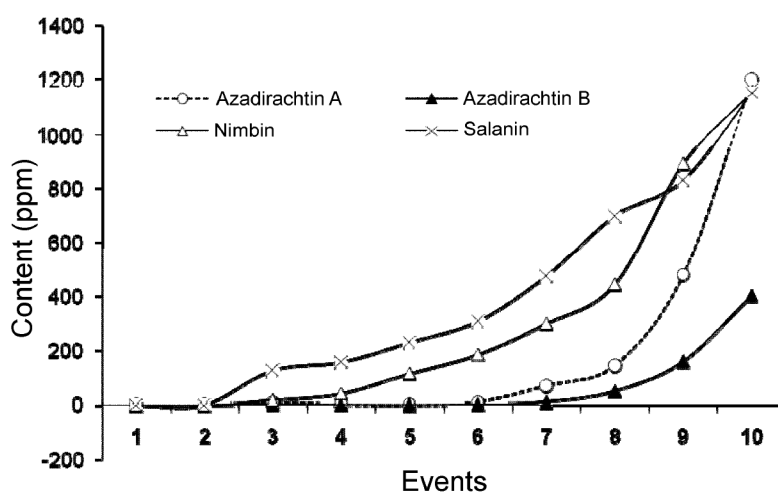
From the results of this study, it is clear that formation of N and S started much earlier than AA and AB. AA and AB formation appeared to be starting actually in green unripe hard fruits (event VI). While AA concentration increased rapidly in ensuing events, AB concentration exhibited relatively much slower increase. At complete fruit maturation or fruit fall stage, on an average, concentration of AA, N and S was more or less similar, while that of AB was one-third of what was obtained for AA, N and S. Yakkudi and coworkers<sup>10</sup> observed that azadirachtin content in *A. indica* fruits (irrespective of trees grown from seeds of varied location) appeared only after 9th week of inflorescence initiation, which coincides with the VI reproductive phenological event in our study and reaches maximum around 17th week, which coincides with X or last reproductive phenological event of our study. Similarly, Rengasamy and Parmar<sup>11</sup> did not find azadirachtin at any flowering stage of *A. indica* and reported its occurrence from green fruit stage. Thus, these

**Table 1.** Reproductive phenological events in selected CPTs of *Azadirachta indica*

Event	Period	Number	Activity
Inflorescence and flower buds formation	Last week of March–early second week of April	I	Extended
Flower bud bursting and early flowering	2nd–3rd week of April	II	Brief
Blossoming	3rd of April–early 2nd week of May	III	Extended
Faded flowers and initiation of fruiting	2nd–early 3rd week of April	IV	Very brief
Tiny green, very hard fruits	3rd week of May	V	Very brief
Increment in fruit size, green unripe hard fruit	Early 4th week–May end	VI	Brief
Initiation of fruit colour change and softening, visible yellow spots on fruits	May end–1st week of June	VII	Brief
Yellow pulpy fruits with less harder seeds	2nd–3rd week of June	VIII	Brief
Bright yellow excessive soft and sweet pulpy fruits	3rd week–June end	IX	Brief
Fully matured fruits, heavy fruit fall, fruit drying on tress	June end–3rd week of July	X	Extended

**Table 2.** Meliacin content (ppm) in different reproductive phenological events in *A. indica* (average across selected CPTs)

Meliacins/phenophase	III	IV	V	VI	VII	VIII	IX	X
Azadirachtin-A	9.0	2.5	6.3	10.4	70.0	146	406	1201
Azadirachtin-B	2.2	2.0	1.5	3.5	16.6	50	160	402
Nimbin	18.0	41.0	108	186	301	444	1293	1161
Salanin	13.0	158	192	278	474	699	830	1207

**Figure 1.** Average meliacin content in different reproductive phenological events in neem (taking all the CPTs as single population).

studies substantiate our finding to a greater extent.

The multiple co-relations among tree morphological features, structural traits of matured fruits and meliacin content in these fruits revealed that out of the 45 character combinations only 3 showed significant positive correlations, whereas 2 exhibited negative correlations. The most important co-relations, i.e. within the meliacins, AA and N exhibited significant positive association suggesting thereby that formation of AA and N had some kind of interrelationship such as increase in content of one compound directly associated with increase in other or vice-versa. Kaushish<sup>12</sup> also observed significant positive association in AA and N content in kernel of mature seeds of *A. indica*.

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