Pesticide Research Journal Vol. 7 (2): 140-148, December, 1995

Azadirachtin-A content of seeds of Neem Ecotypes in relation to the Agroecological Regions of India

Seeni Rengasamy and Balraj Singh Parmar
Division of Agricultural Chemicals, Indian Agricultural Research Institute, New Delhi 110 012, India

The azadirachtin-A content of the seed kernels of the neem, *Azadirachta indica* A.Juss, ecotypes from 11 of the 21 agroecological regions of India has been investigated in relation to the agroecological-factors such as ecosystem and climate, growth period (the period of continuous plant growth in a year) and soil. The azadirachtin -A content ranged from 0.14 -2.02% w/w of kernel with an average of 0.68%. The ecotypes growing in the regions with coastal, arid and semiarid ecosystems showed high (>0.72%) and those from the regions with subhumid ecosystem showed very low (0.27%) average azadirachtin -A contents. The growth period upto 150 d appeared to be more conducive for azadirachtin -A content under arid and semiarid ecosystems. The ecotypes growing in the agroecological regions with red soil had higher azadirachtin -A content as compared to those growing in other soil types; the highest azadirachtin -A content being recorded in the samples from red soil falling under the rain-shadow areas of south-west monsoon.

Key words : Azadirachtin -A, neem, *Azadirachta indica*, agroecological regions

Environmental pollution hazard caused by the synthetic pesticides prompted the world to look for environment friendly pesticide products. Neem (*Azadirachta indica* A.Juss) is emerging as a safe alternative botanical source of such products. It is a cross pollinated deciduous tree with wide genetic variability and grows in all parts of India.

The pest control property of neem has been attributed to its chemical component azadirachtin -A in the seed kernel[4,5]. Neem ecotypes of Asian and African origin have been reported to show a significant variation in the azadirachtin -A content of the seeds[1,2,6]. However, the influence of the agroecological factors on its content has not been reported. Present study is a step in this direction.

India is divided into 21 agroecological regions, characterized by their predominant climate, growth period (GP) i.e. the period of continuous plant growth in a year, and soil type. These regions are grouped into 6 ecosystems namely: arid, semiarid, hot humid-perhumid, subhumid, coastal and island[3]. In this paper, the azadirachtin-A content of 42 seed samples from 11 agroecological regions of India (Fig. 1, Table 1) spreading over a latitude range of 8 - 32^{0}N and longitude range of 68 - 90^{0}E is reported. A correlation of its content with the agroecological features of the regions has been attempted.

MATERIALS AND METHODS

Materials

Neem seed samples were obtained either through M/s Neem Mission, Pune, India or collected directly from the specified places. The regionwise distribution of samples alongwith the features of each region are given in Table 1. A standard azadirachtin -A sample from M/s Trifolio-M GMBH, Germany, was obtained through the courtesy of Mr.C.M.Ketkar, Neem Mission, Pune, India. Laboratory grade reagents and solvents were used in the extraction and

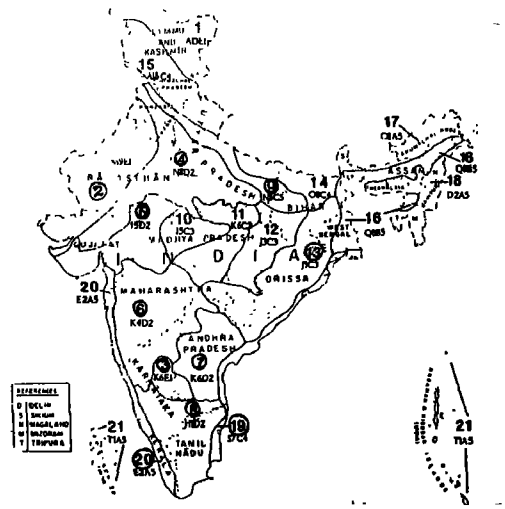

Fig. 1. Agroecological regions of India[3] (**Arid Ecosystem**: 1. Western Himalayas 2. Western Plain and Kutch Peninsula 3. Deccan Plateau. **Semiarid Ecosystem**: 4. Northern Plain and Central Highlands 5. Central (Malwa) Highlands and Kathiawar Peninsula 6. Deccan Plateau 7. Deccan Plateau and Eastern Ghats 8. Eastern Ghats (TN Uplands) and Deccan Plateau. **Subhumid Ecosystem**: 9. Northern Plain 10. Central Highlands (Malwa and Bundelkhand) 11. Deccan Plateau and Central Highlands (Bundelkhand) 12. Eastern Plateau (Chhatisgarh) 13. Eastern (Chhotanagpur) Plateau and Eastern Ghats 14. Eastern Plain 15. Western Himalayas. **Humid Perhumid Ecosystem**: 16. Assam and Bengal Plains 17. Eastern Himalayas 18. North Eastern Hills (Purvanchal). **Coastal Ecosystem**: 19. Eastern Coastal Plains 20. Western Ghats and Coastal Plains 21. Islands of Andaman-Nicobar and Lakshdweep.[Samples were analyzed from the encircled regions.]

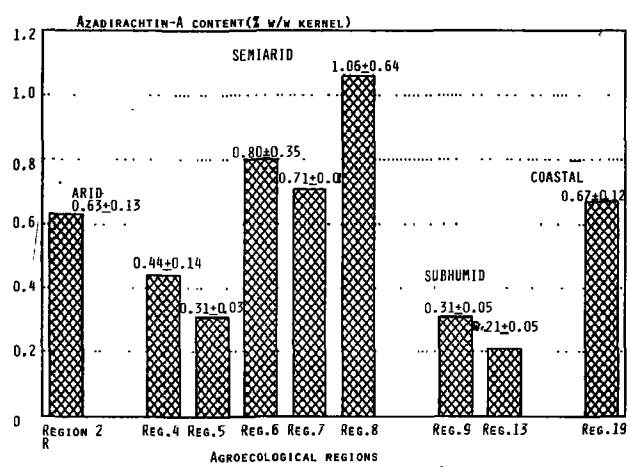

Fig. 2. Average azadirachtin-A content of neem ecotypes from different agroecological regions

Table 1 : Distribution of neem test samples among the agroecological regions of India

Ecosystem	Reg.no.	Region	Climate	Soil	Growth period	No. of samples
Arid	2	Western Plain & Kutch Peninsula	Hot arid	Desert and saline	<90	2
	3	Deccan Platteau	Hot arid	Red, black	<90	1
Semiarid	4	Northern Plain & Central Highlands	Hot semiarid (coldwinter)	Alluvium and derivatives	90-150	8
	5	Central (Malwa) Highlands & Kathiawar Peninsula	Hot semiarid (cold winter)	Medium deep black	90-150	3
	6	Deccan Plateau	Hot semiarid (mild winter)	Shallow medium black	90-150	7
	7	Deccan Plateau & Eastern Ghats	Hot semiarid (mild winter)	Red & black	90-150	2
	8	Eastern Ghats (TN Uplands) & Deccan Plateau	Hot semiarid(mild winter)	Red loamy	90-150	9
Subhumid	9	Northern Plain	Hot subhumid	Alluvium derived	90-150	4
	13	Eastern Plateau (Chota Nagpur) & Eastern Ghats	Hot subhumid	Red loamy	150-180	2
Coastal	19	Eastern Coastal Plains	Hot subhumid coastal	Alluvium derived	150-210	3
	20	Western Ghats, Coastal Plains	Hot subhumid coastal Hot, humid, perhumid	Red lateritic & alluvium derived	>210	1

analytical grade solvents for High Performance Liquid Chromatographic (HPLC) analysis.

Methods

The seed samples were cleaned, manually decorticated and the kernels were powdered using a waring blender. The powder (0.5 g) was extracted by refluxing with ethanol (3 x 20 ml, 30 min each). The contents were cooled and filtered through a G2 septum fitted Buchner funnel collecting the filtrates in the same flask and made upto 100 ml with ethanol. Aliquots were analyzed for the azadirachtin -A content by HPLC using a Shimadzu HPLC system fitted with LC9A pumps in binary mode, Rheodine 7161 injector with 20 µl loop, Shim Pack CLC-phenyl stainless steel column (6 mm id x 15 cm) and SPDM6A photodiode array detector. The operating conditions were : mobile phase, methanol-water (65:35) at 1.0 ml min^{-1}; detector wavelengths, 214 and 250 nm, sensitivity, 0.1 AUFS. The data were acquired in a PCS-DG India Ltd workstation and quantification was done in the post analysis session at 214 nm. Retention time for azadirachtin -A was 8.33 min. After every four injections the column was cleaned by gradient methanol-water elution. Imperfect cleaning led to erratic column behaviour.

RESULTS AND DISCUSSION

Azadirchtin-A content of neem ecotypes

Azadirachtin-A content of the seed kernels is reported in Table 2. It ranged from 0.14 to 2.02 % (w/w, kernel basis), the average being 0.68± 0.45 %. A wide variation in the azadirachtin-A content of seed kernels has been observed.

The average azadirachtin-A contents for the regions under study are given in Fig. 2. The highest average was observed in the region 8 and the lowest in the region 13. The sample with the highest azadirachtin-A content (S-32, 2.02%) was observed in the region 8 and that with the lowest azadirachtin -A content (SMT 5, 0.14%) was in the region 4 (Table 2). The sample standard deviation was the highest in region 8 (± 0.64). This is a larger region as compared to others with differences in soil type and rainfall during south-west and north-east monsoons. The samples S15, S16, S25 and S32 from the region 8 contained more azadirachton-A than the regional average of 1.06%. These samples were from the rain shadow area of south-west monsoon. The rainfall by south-west monsoon that coincides with the flowering and fruit setting in neem trees is not much in this area. Only one sample each was analyzed from the regions 3 and 20, preventing any worthwhile conclusion.

Azadirachtin-A content of the ecotypes *vis-a-vis* the features of agroecological regions

Ecosystem and climate : The test samples represented 4 (arid, semiarid, subhumid and coastal) of the 6 ecosystems. No sample was drawn from the hot humid - perhumid and island ecosystems. The highest average azadirachtin-A content (0.90 ± 0.37%) was recorded in samples from the coastal and the lowest from subhumid ecosystems (Fig. 3A). The former comprised of regions 19

Table 2. Azadirachtin content of neem ecotype from different agroecological regions

Region no.	Sample no.	Azadirachtin content of kernel (% w/w)	Regional average (% w/w)
2	S-27	0.72	0.63 ± 0.13
	S-29	0.54	
3	S-13	0.99	0.99
4	S-21	0.46	
	S-24	0.47	
	S-28	0.57	
	SMT-1	0.41	
	SMT-2	0.42	
	SMT-5	0.14	
	H-1	0.51	
	RPS	0.53	0.44 ± 0.14
5	S-4	0.31	
	S-18	0.28	
	S-30	0.33	0.31 ± 0.03
6	S-1	0.61	
	S-2	0.65	
	S-3	1.27	
	S-8	1.21	
	S-10	0.27	
	S-14	0.79	
	S-22	0.71	0.80 ± 0.35
7	S-17	0.70	
	S-26	0.72	0.71 ± 0.01
8	S-7	0.49	
	S-9	0.29	
	S-12	0.46	
	S-15	1.49	
	S-16	1.66	
	S-25	1.67	
	S-32	2.02	
	S-34	0.74	
	CS	0.74	1.06 ± 0.64
9	S-31	0.30	
	SMT-3	0.38	
	SMT-4	0.30	
	SMT-6	0.24	0.31 ± 0.06
13	S-23	0.25	
	S-33	0.17	0.21 ± 0.05
19	S-6	0.56	
	S-19	0.80	
	S-20	0.73	0.67 ± 0.12
20	S-11	1.52	1.52

Overall average 0.68

Sample standard deviation ± 0.45

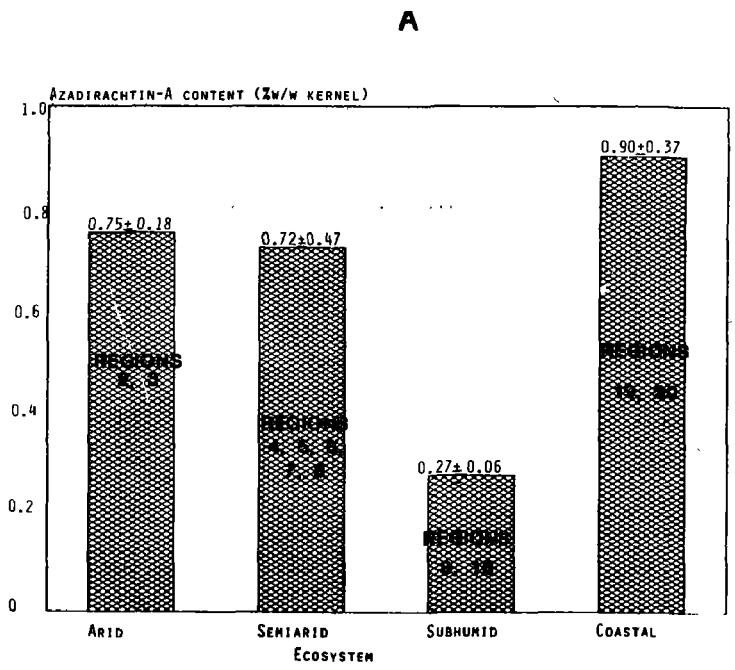

Fig. 3a. Azadirachtin-A content of neem ecotypes in relation to ecosystems

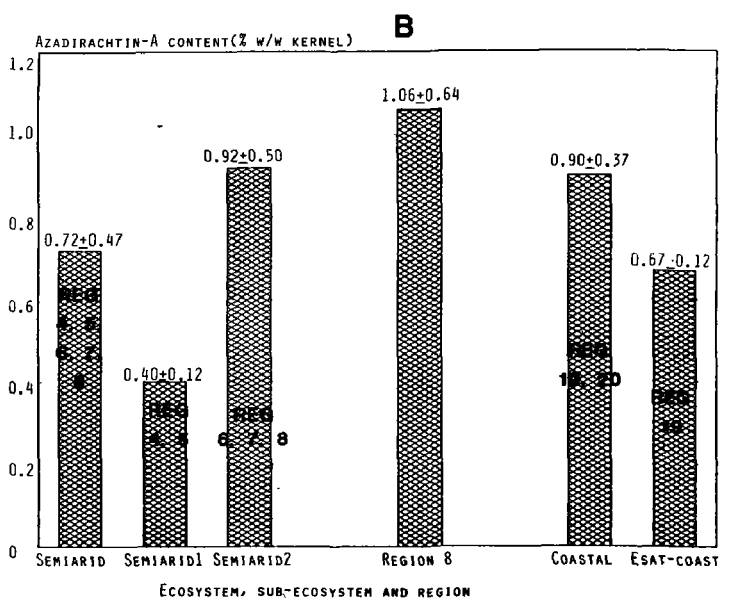

Fig. 3b. Azadirachtin-A content of neem ecotypes in relation to ecosystem, subecosystem and region

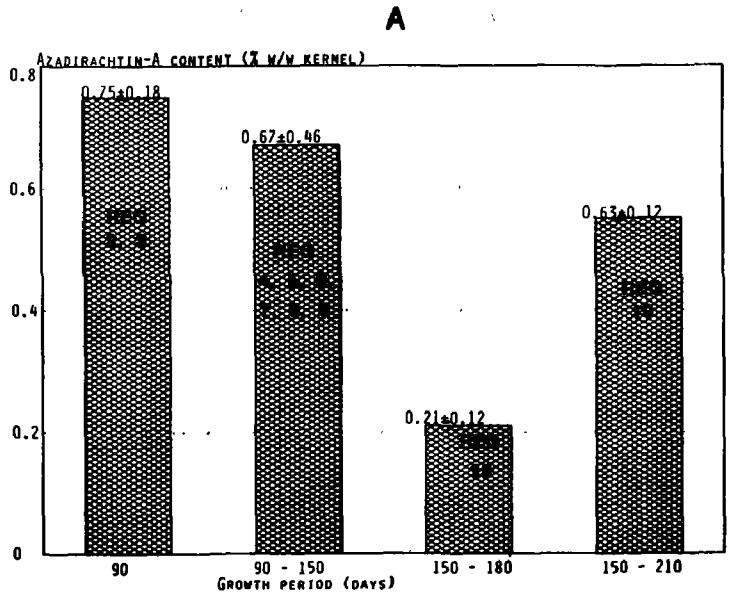

Fig. 4a. Azadirachtin-A content of neem ecotypes in relation to growth period

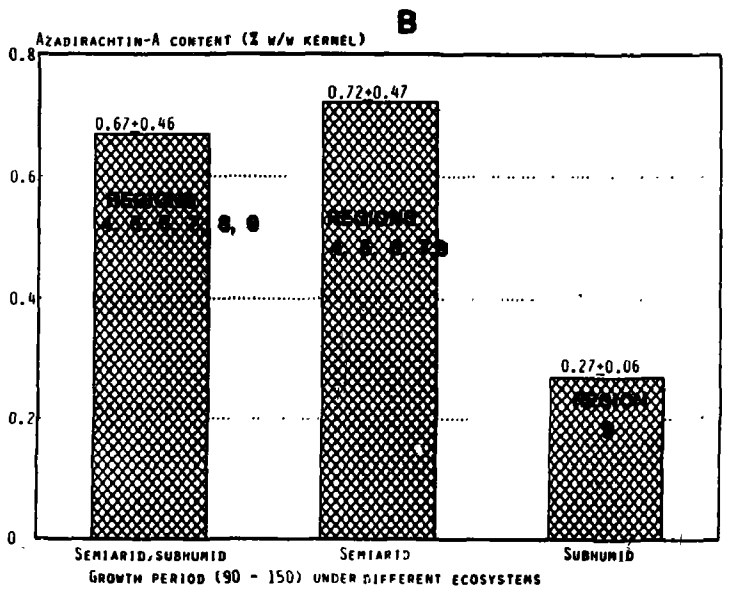

Fig. 4b. Azadirachtin-A content of neem ecotypes in relation to climate under the growth period 90- 150 days

and 20. The average content of azadirachtin-A for this ecosystem was 0.90 ± 0.37%. Only one sample was analyzed from the region 20 (west coast) and the average content for region 19 (east coast, Fig. 3B) was 0.67 ± 0.12%. This average was less than that of the arid and semiarid ecosystems.

The samples from the arid ecosystem (regions 2 and 3) recorded the second largest average content of azadirachtin-A. There is relatively less heterogeneity in this ecosystem as evidenced by a low SD of ± 0.18. Only one sample was analyzed from region 3 which recorded a content of 0.99%. Two samples were analyzed from Region 2, the average of which was 0.63 ± 0.13%.

It is apparent from Fig. 3A that the sample heterogeneity was very high among the samples from the semiarid and coastal ecosystems. Semiarid ecosystem comprises of the agroecological regions 4, 5, 6, 7 and 8. Among these, the regions 4 and 5 have cold winter and the regions 6, 7 and 8, a warm winter. The samples from the semiarid regions 4 and 5, with cold winter, (Semiarid 1, Fig. 3B) recorded low average azadirachtin-A content (0.40 ± 0.12%) as compared to those in the semiarid regions 6, 7 and 8 with warm winter (Semiarid 2, Fig. 3B). Among the regions 6, 7 and 8 under Semiarid 2, the region 8 recorded the highest average azadirachtin-A content (1.06 ± 0.64%) as compared to the regions 6 and 7 (Fig. 3B).

In general, samples from the ecotypes growing in the coast al, arid and semiarid ecosystems had relatively higher azadirachtin-A contents. Ecotypes of some semiarid regions of South-Indian Peninsula recorded relatively higher averages as compared to the averages for those of coastal and arid ecosystems.

Growth period: The growth period (GP) depends largely on the climate and the moisture retaining capacity of the soil and it affects the physiological functioning of the plant. The regions 2 and 3 under arid ecosystem have GP less than 90 days. All the regions studied under semiarid ecosystem (regions 4-8) and region 9 under subhumid ecosystem have GP 90 - 150 days. The regions 13, 19 and 20 have GP regimes 150 - 180, 150 - 210 and >210 d respectively.

The azadirachtin-A content of the ecotypes growing in different growth period regimes is given in Fig. 4A. The ecotypes growing in the regimes of <90, 90-150 and 150-210 d showed higher and comparable average azadirachtin-A contents. Those growing under 150-180 d GP regimes showed below average azadirachtin-A content. This may be because this regime lies under subhumid ecosystem.

Wide sample heterogeneity was found in the 90-150 d GP regime (regions 4-9) as compared to other regimes (Fig. 4A). The regions 4,5,6,7, and 8 fall under the semiarid ecosystem and the region 9 under the subhumid ecosystem. The azadirachtin- A content appears to be adversely affected by the hot sub-humid climate under the GP of 90-150 d (Fig. 4B). Relatively less, though still considerable, sample heterogeneity was found among the ecotypes growing in the GP regime <90 d though it lies in one ecosystem (arid, agroecological regions 2 and 3). This is due to the location of the agroecological regions *vis-a-vis* region 2 in the Northern Plain and region 3 in the Great Peninsular Plateau. The GP regimes 150-180 and 150-210 d were found in single agroecological region 13 under subhumid ecosystem and 19 under coastal ecosystem respectively and showed relatively less sample heterogeneity.

In general the growth periods up to 150 d under arid, and semiarid ecosystems appeared to be more conducive for high azadirachtin-A content.

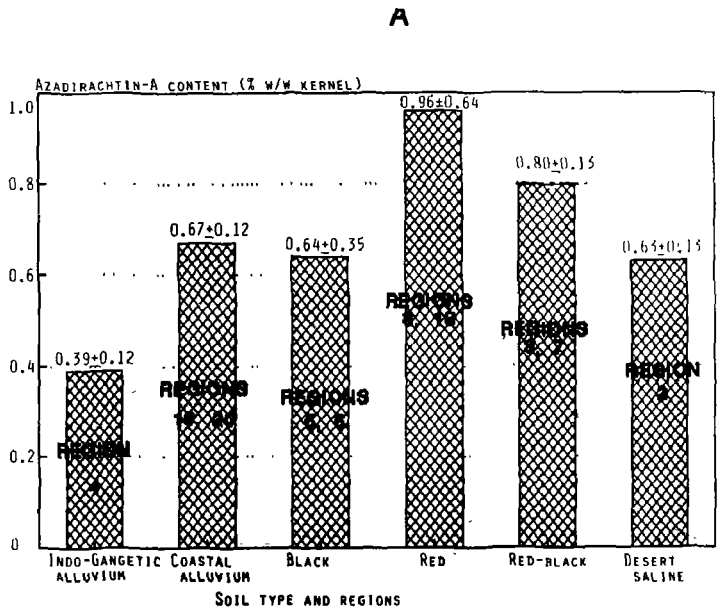

Fig. 5a. Azadirachtin-A content of neem ecotypes in relation to soil type

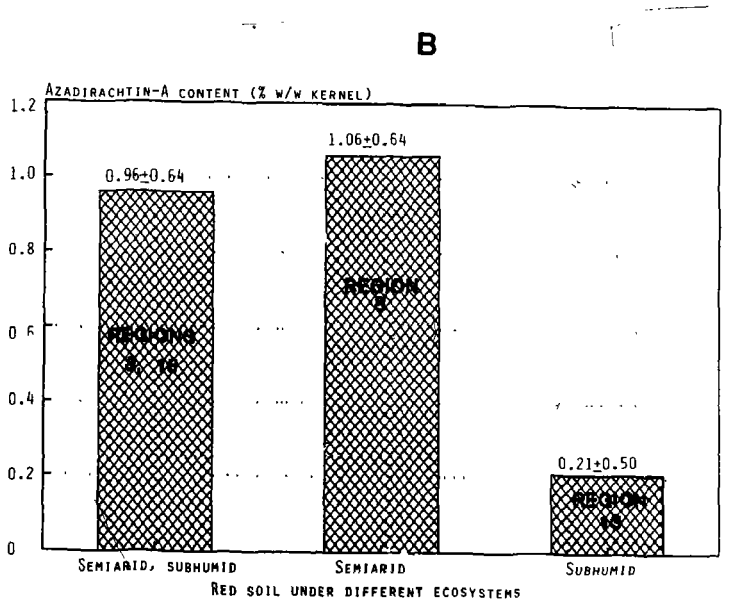

Fig. 5b. Azadirachtin-A content of neem ecotypes in relation to red soil under semiarid and subhumid climates

Soil type: Azadirachtin-A content of samples from different soil types is given in Fig. 5A. Seeds from red soils contained the highest average azadirachtin-A content (0.96 ± 0.64%) and those from the Indo-Gangetic alluvium soils contained the lowest (0.39± 0.12%). Samples from the black, desert and saline soils or coastal alluvium had comparable azadirachtin-A contents. The samples from red black soil ranked next to the red soil in azadirachtin-A content and it was above the average value of 0.68%.

The samples from red soils showed the highest sample heterogeneity in their azadirachtin-A content, because these soils occurred in two regions 8 and 13, under two ecosystems namely semiarid and subhumid (Fig. 5B). The samples from red soil under semi arid climate had higher azadirachtin-A content as compared to those from sub humid climate. The seeds from the black soil also showed considerable heterogeneity in their azadirachtin-A content. This may be because the black soils occurred in two different regions 5 and 6 though under the same (semiarid) ecosystem. Samples from Red-Black soils showed relatively less sample heterogeneity in their azadirachtin-A content though these soils occurred in two ecosystems - arid (region 3) and semi-arid (region 7).

Generally, the ecotypes growing in red soils under semiarid climate contained the highest azadirachtin-A content.

ACKNOWLEDGEMENT

This study was carried out as a part of the A.P. Cess Fund Scheme (C-1/90/93 IAR-H10/2800). The financial assistance from the Indian Council of Agricultural Research is gratefully acknowledged.

REFERENCES:

1. Ermel K, Pahlich E and Schmutterer H (1984) Comparison of the azadirachtin content of neem seeds from ecotypes of Asian African origin. *Proc 2nd Int Neem Conf*, Rauischholzhausen, West Germany, p. 91 -94.
2. Ermel K, Pahlich E and Schmutterer H (1987) Azadirachtin content of neem kernels from different geographical locations and its dependence on temperature, relative humidity and light. *Proc 3rd Int Neem Conf*, Nairobi, Kenya 1986. p. 185 -194.
3. NBSS and LUP (1990) *Agroecological regions of India*. Bull. 24. National Bureau of Soil Survey and Land Use Planning, Nagpur, India.
4. Rembold H (1988) Isomeric azadirachtins and their mode of action, In: *Focus on Phytochemical Pesticides* Vol 1: *The Neem Tree*. (Ed.M. Jacobson) CRC Press, Inc. Boca Raton, Florida. p. 47 - 57.
5. Rembold H, Forster H, Czoppelt Ch, Rao P J and Sieber K P (1983) The azadirachtins, group of insect growth regulators from the neem tree, In: *Natural Pesticides from the Neem tree and other Tropical Plants*. Proc. 2nd Int. Neem Conf. Rauischholzhuasen, 1983 (Eds. H.Schmutterer and K.R.S. Ascher) P. 153-162. GTZ, Eschborn FRG.
6. Rengasamy S, Kaushik N, Jitendra Kumar, Kaul O and Parmar B S (1993) Azadirachtin content and bioactivity of some neem ecotypes of India. *Proc World Neem Conf* Oxford and IBH Co. Pvt. Ltd., New Delhi (in press). Abstract in *Abstracts World Neem Conference*, 24 - 28 Feb. 1993, Bangalore, India, p.3.

Manuscript received September 26, 1995, accepted October 30, 1995.