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EXTRACTION OF NEEM OIL FROM NEEM SEEDS OF DIFFERENT GEOGRAPHICAL REGIONS OF INDIA BY TWO PROCESSES AND STUDY OF THEIR PROPERTIES FOR STANDARDIZATION OF PARAMETERS USEFUL FOR COATING OF UREA FERTILIZER

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ABSTRACT

Neem oil has an extensive history of human use in India and surrounding regions for a variety of therapeutic purposes. Neem oil is not used for cooking purposes. However, it is used for preparing cosmetics (soap, hair products, body hygiene creams, hand creams) and in Ayurvedic and Unani medicine, in the treatment of a wide range of afflictions. The most frequently use in skin diseases, inflammations, fevers and rheumatic disorders etc. Neem oil is not known to be harmful to mammals, birds, earthworms or some beneficial insects if it is not concentrated directly into their area of habitat or on their food source. Formulations made of neem oil also find wide usage as a biopesticide for organic farming, as it repels a wide variety of pests.

KEYWORDS: Neem Oil, biopesticide, urea, seed, fertilizer, organic farming.

INTRODUCTION

Neem oil is obtained by cold-pressing neem seeds followed by extraction with organic solvents like n-hexane. Neem Oil or Neem Seed Oil is a Brownish Yellow color Liquid, with smell of Garlic. Neem Oil is slightly soluble in water and has 6.5 to 7.5 pH value, it boils at more than 200˚C and freeze at 13˚C. It is mainly a composition of triglycerides and contains many triterpenoid compounds, which are responsible for its bitter taste. Azadirachtin is a known triterpenoid in neem oil. The azadirachtin content in neem oil is found upto 2500ppm depending on the extraction technology and quality of the neem seeds crushed. Nimbin, Salannin and many other triterpenoid have been credited with some of neem oil's properties as an antiseptic, antifungal, antipyretic and antihistamine. N eem oil also contains several sterols, including (campesterol, beta-sitosterol, stigmasterol). The coating of urea with neem oil or other neem products found a great favour by Indian researchers and farmers as it is much cost effective way to save the N fertiliser.

Many research studies in India have conclusively established that neem oil acts as an effective nitrification inhibitor if coated onto urea.

Keeping in mind the following main benefits, Government of India has made it mandatory to coat entire quantity of Fertilizer Grade Urea with Neem Oil:

- Prevent diversion of Urea
- Reduce the quantity of Urea required in the fields by 10% - 15% by slowing down the process of nitrification.
- Increase the crop yield
- Provide pesticidal benefits of Neem oil to the fields

Neem oil characteristics prescribed by BIS-4765 are shown in Table 1.

Table 1: Characteristics of Neem Oil as per BIS 4765 – 1975.

No.	Characteristic	Specification	
		Neem kernel oil	Depulped neem seed oil
1	Moisture & insoluble impurities, % by mass	0.3 max	0.5 max
2	Refractive index at 40 deg C	1.4615 to 1.4705	1.4615 to 1.4705
3	Saponification value	180 to 205	175 to 200
4	Iodine value	65 to 80	65 to 80
5	Acid value	15 max	20 max
6	Unsaponifiable matter, % by mass	2 max	2 max
7	Titre, deg C	36 min	36 min

Neem oil coating is carried out on Fertilizer grade Urea in India (both indigenously produced and imported) at an approximate level of 0.5 – 0.7 kg per Tonne of Urea, so as to meet with FCO (Fertilizer Control Order) prescribed limit of 0.035% min coating of Neem oil on Urea. This policy change has created increased demand of Neem Oil in India. Criteria fixed up in FCO for Neem Oil quality are given in Table 2.

Table 2: Characteristics of Neem Oil prescribed in Fertilizer Control Order.

No.	Characteristic	Specification
1	Moisture & insoluble impurities, % by mass	1.0 max
2	Specific gravity at 30 deg C	0.85 to 0.95
3	Iodine value	65 to 95
4	Saponification value	160 to 205
5	Azadirachtin content, ppm	150 minimum

Because it is possible to meet above criteria by adding Technical Grade Azadirachtin concentrate in other vegetable oils, these specifications for quality check of neem oil are not sufficient to safeguard neem oil quality with respect to mal-practices / adulteration with other oils. There is a great need to study neem oil characteristics prevailing in country to decide suitable characteristics and their limits to ensure coating of Urea by pure neem oil. This paper describes quality of pure neem oil obtained by processing neem seeds collected from several states of India.

MATERIALS

[A] Neem Seeds: Neem seeds from 09 different geographical regions (Table 3) were collected in the month of April, 2017. Since the seed collection period of Neem seeds is between May – July, the seeds collected in April 2017 were of previous year (2016), hence were over dried.

Table 3: Details of Neem seeds collected from different geographic regions in April, 2017 (from available stock of seeds collected in during May – July, 2016) & processed under expeller & solvent extraction.

Area-wise Neem seed collection			Oil recovery process	Oil collected	Oil sample code
Area	State	Seed quantity			
Hyderabad (HYD)	Andhra Pradesh (AP)	25 kg	Expeller	Nil	-
			Solvent extraction	3.45 kg	S1
Jahabua (JHB)	Madhya Pradesh (MP)	25.5 kg	Expeller	200 ml	E8
			Solvent extraction	2.5 kg	S2
Jambusar (JBS)	Gujarat (GJ)	28 kg	Expeller	Nil	-
			Solvent extraction	2.15 kg	S3
Dhulia (DHU)	Maharashtra (MH)	26.2 kg	Expeller	200 ml	E7
			Solvent extraction	2.45 kg	S4
Disha (DSH)	Gujarat (GJ)	24.5 kg	Expeller	75 ml	E1
			Solvent extraction	2.35 kg	S5
Kanpur (KNP)	Uttar Pradesh (UP)	27 kg	Expeller	200 ml	E5
			Solvent extraction	3.5 kg	S6
Udaipur (UDZ)	Rajasthan (RJ)	25.5 kg	Expeller	150 ml	E6
			Solvent extraction	4.1 kg	S7
Kappal (KPL)	Karnataka (KA)	24.5 kg	Expeller	25 ml	E9
			Solvent extraction	3.05 kg	S8
Kapadwanj (KPW)	Gujarat (GJ)	20 kg	Expeller	Nil	-
			Solvent extraction	2.1 kg	S9

These seeds were individually processed through Expeller unit (M/s Aqua Safe Mine, Savli, Gujarat) for deriving as much oil as possible. Due to over-drying of seeds, very meager quantity could be recovered from Expeller process (Table 3). In some of the cases, no oil could be recovered & dry powder came out of the expeller, instead of oiled cake.

Thereafter, the oiled cakes / powder (collected from expeller unit) were processed through solvent extraction (M/s Ahepra Health Care, Ankleshwar, Gujarat) using n-hexane to derive neem oil. The process was similar to Soxhlet Extraction method. Oil was made hexane-free through distillation process. Expected recovery of oil could be collected from this process.

The study of expelled and solvent extracted Neem Oil samples was conducted for Physical characteristics, Chemical tests and Active ingredients.

[B] Neem Oil from Large Scale manufacturing plant (Expeller grade & Solvent extracted): Alongwith samples mentioned in Table 3, below mentioned composite Neem Oil samples, collected from large scale

operation of Expeller & Solvent Extraction processes in 2016 season, were also studied as reference and compared.

Areas from where major quantity of Neem seeds was obtained	State	Oil recovery process	Oil sample code
North Gujarat, Banaskantha, Dahod	Gujarat	Expeller	L1
		Solvent extraction	L2

[C] Other vegetable oils: Other vegetable oils like Cotton Seed oil, Rice bran oil, Transformer (Mineral) oil, Karanj oil & Palm oil were collected from open market or oil industry. These being cheap oils having alike properties were studied to judge their potential for blending with Neem oil sold at higher rate.

[D] Azadirachtin Powder: In order to evaluate effect of addition of Azadirachtin rich materials in Neem Oil, 02 Samples of Azadirachtin Technical grade as well as 02 samples of market sold Neem Based Insecticides (Table 4) were also studied for presence of limonoids like Azadirachtin, Nimbin and Salannin as below:

Table 4: Azadirachtin rich materials

Azadirachtin source	Sample code
Azadirachtin Tech - 35% pure powder	T1
Azadirachtin Tech - 4% solution in n-butanol	T2
Neem Based Insecticide containing 1% Azadirachtin	I1
Neem Based Insecticide containing 5% Azadirachtin	I2

METHODOLOGY

The analytical methods were followed as prescribed in various Standards (IS 548 part I, 1964) & Manuals for parameters like Sp Gravity, Moisture, Refractive index, Colour, Pour Point, Titre, Flash point, Viscosity, Acid value, Iodine number, Saponifaction number, Unsaponifiable matter, Azadirachtin. The analysis method for Nimbin and Salannin was established by HPLC in line with Azadirachtin analysis. The standard reference materials, wherever necessary, were procured from reputed manufacturers having above 90% purity.

RESULTS AND DISCUSSION

Data presented in Tables 5 & 6 for various parameters other than limonoids for Pure Neem Oil samples, derived through expeller & solvent extraction processes, exhibit that the characteristics are similar to those of many other vegetable oils & fats. Because of this reason, it is not possible to identify presence of other oil in Neem Oil by these physico – chemical tests. Therefore, limits are always prescribed by national & international standards for determining the genuineness of such materials.

The variations in Analytical Results presented in Table 5 for various parameters for Solvent Extracted Pure Neem Oil samples show that these tests might not be useful for fixing acceptance criteria for Neem Oil. However, moisture and insoluble matter is an important parameter to be taken into consideration for checking its contamination with undesirable components. Therefore, FCO prescribes its limit as 1% max.

Table 5. Analysis data of Neem oils samples S-1 to S-9, E-1 to E-9, L1, L2 for various parameters.

Area of state	State	Sample code	Sp gr *at 30^{0}C*	Ref. Index *at 20^{0}C*	Color *Lovi-bond*	PP ^{0}C	FP ^{0}C	Visco-sity *cst*	Titre ^{0}C	M %	MIB %
HYD	AP	S1	0.9503	1.4820	55.5	6	151	80	33	0.25	<0.1
		E4	Not analysed as sample quantity less								
JHB	MP	S2	0.9452	1.4760	42.1	1	100	46.6	32	0.16	<0.1
		E8	Not analysed as sample quantity less								
JBS	GJ	S3	0.9367	1.4790	79.92	19	144	55.63	33	0.30	<0.1
		E3	Not analysed as sample quantity less								
DHU	MH	S4	0.9301	1.4810	75.06	6	158	73.47	34	0.23	<0.1
		E7	Not analysed as sample quantity less								
DSH	GJ	S5	0.9570	1.4750	77.04	5	184	49.25	32	0.29	<0.1
		E1	Not analysed as sample quantity less								
KNP	UP	S6	0.9567	1.4820	86.4	4	146	60.88	33	0.14	<0.1
		E5	Not analysed as sample quantity less								
UDZ	RJ	S7	0.9317	1.4820	80.12	6	174	55.74	34	0.10	<0.1
		E6	Not analysed as sample quantity less								
KPL	KA	S8	0.9218	1.4730	72.8	1	133	47.53	34.5	0.13	<0.1
		E9	Not analysed as sample quantity less								
KPW	GJ	S9	0.9457	1.4590	94.6	2	156	19.6	33	0.31	<0.1

		E2	Not analysed as sample quantity less							
Large Scale Production - Expeller		L1	0.9480	1.4773	66	18	179	74.47	33.2	0.34	<0.1
Large Scale Production - Solvent extraction		L2	0.9539	1.4812	69	18	209	51.23	34.6	0.25	<0.1
Min			0.9218	1.4590	42.1	1	100	19.6	32	0.1	<0.1
Max			0.9570	1.4820	94.6	19	209	80	34.6	0.34	<0.1
Avg			0.9434	1.4770	72.6	8	158	55.9	33.3	0.23	<0.1

"PP – Pour point, FP – Flash point, MIB – Material insoluble in Benzene
HYD – Hyderabad, JHB – Jahabua, JBS – Jambusar, DHU – Dhulia, DSH – Disha, KNP – Kanpur, UDZ – Udaipur, KPL – Kappal, KPW – Kapadwanj

***Table 6. Analysis data of Neem oils samples S-1 to S-9, E-1 to E-9, L1, L2 for important* oil parameters & Neem limonoids.**

Area of state	State	Sample code	Acid value	Sap. number	Iodine value	Un-sap. matter	Aza	Nimbin	Salannin
			mg KOH/gm	*mg KOH/gm*	*mg I_2/ gm*	*%*	*ppm*	*ppm*	*ppm*
HYD	AP	S1	11.3	178.2	78.4	2.09	641	2533	3729
		E4	-	-	-	-	-	-	-
JHB	MP	S2	4.0	177.2	77.9	1.82	168	1170	1881
		E8	3.7	178.7	79.8	2.4	398	1437	2023
JBS	GJ	S3	43.9	183.4	76.7	2.17	61	3846	4728
		E3	-	-	-	-	-	-	-
DHU	MH	S4	5.2	179.3	80.6	2.33	190	2145	2266
		E7	10.4	181.4	81.7	2.49	316	3361	1591
DSH	GJ	S5	9.5	175.9	81.2	2.6	95	2784	2147
		E1	6.2	180.3	86.4		379	1467	1381
KNP	UP	S6	11.7	179.3	81.7	1.97	166	2298	2311
		E5	5.4	184.1	81.2	2.71	531	2595	2151
UDZ	RJ	S7	8.6	178.6	84.0	2.52	218	2991	3448
		E6	7.4	186.2	79.7	--	365	1377	1793
KPL	KA	S8	7.3	174.3	85.0	2.65	89	1585	4134
		E9	--	--	--	--	378	--	--
KPW	GJ	S9	49.3	181.4	84.8	1.87	68	2141	3268
		E2	--	--	--	--	--	--	--
Large Scale Production - Expeller		L1	6.5	174.0	69.0	1.96	441	2953	3665
Large Scale Production - Solvent extraction		L2	7.9	172.5	77.8	2.51	70	5217	5366
Min			3.7	172.5	69.0	1.82	61	1170	1381
Max			49.3	186.2	86.4	2.71	641	5217	5366
Avg			12.4	179.0	80.4	2.29	269	2494	2868

HYD – Hyderabad, JHB – Jahabua, JBS – Jambusar, DHU – Dhulia, DSH – Disha, KNP – Kanpur, UDZ – Udaipur, KPL – Kappal, KPW – Kapadwanj

Acid Value: Acid value (Table 6) in majority of the samples is found below 12, whereas samples from Jambusar (Guj) & Kapadwanj (Guj) are having acid value of 43.9 & 49.3 resp. Such high value may be because of the aging of the seeds, as acid value increases with age of seed / oil due to formation of free fatty acid.

Saponification no & Iodine no: The results of Saponification no and Iodine value (Table 6) have ranged from 172.5 – 186.2 & 69 – 86.4 resp. in pure Neem Oil samples of both Expeller process & Solvent process. The result bands are narrower than allowable ranges as per BIS & FCO. Hence, by narrowing the prescribed limits, there is a possibility of using these tests as some of the governing criteria.

Unsaponifiable matter: Unsaponifiable matter (Table 6) in all these Pure samples has ranged from **1.82 to 2.71 %.** This parameter can be a check point for assessing presence of mineral oil in Neem Oil. Though BIS has specified max 2% unsaponifiable matter in Neem Oil, the present study indicates that limit of 2% is not achievable. The same needs to be revised upward to a level of **4% max**.

Azadirachtin: The analysis data (Table 6) shows that all **Expeller** grade neem oil samples are having **> 300 ppm** Azadirachtin, whereas the same is found quite low in solvent extracted neem oils. **4 out of 10** samples have shown Azadirachtin **<100 ppm,** lowest being **61 ppm**. The observations made during present study indicates that it might not be possible for manufacturing industry to achieve FCO prescribed limit of Azadirachtin (150 ppm min) for Solvent (n-Hexane) Extracted Pure Neem Oil samples on continuous basis, though they are unadulterated. It is also evident from data that bare minimum Azadirachtin level of 70 – 90 ppm seems achievable through solvent extraction process.

Additional Neem Limonoids: The study of additional Neem limonoids like Nimbin & Salannin holds good promise to improve neem oil's quality criteria. The oil recovered on large scale in year 2016 show **Nimbin concentration of 2953 & 5217 ppm** in Expeller & Solvent Extraction process resp. Salannin concentration in these samples is found **3665 & 5366 ppm** resp. These data exhibit that Nimbin & Salannin are better extracted with n-Hexane, rather than Expeller. These data also indicate that Nimbin level above 2500 ppm and Salannin level above 5000 ppm are achievable in pure Neem Oil on large scale.

Nimbin: Nimbin concentration in oils derived specifically for present study is found **>2100 ppm in 10 out of 14 samples** and near 1400 ppm in 3 out of 14 samples. Taking commercially produced Neem Oil (L1, L2) into consideration, average value of 16 samples is worked out to be 2494 ppm Nimbin. These observations indicate that it could be possible for Neem Oil manufacturers to achieve **2500 ppm Nimbin** on an average during large scale oil recovery using Neem seeds of the same season.

Salannin : Similarly, Salannin concentration is found **>2000 ppm in 10 out of 14** samples and 1591 – 1881 ppm in 3 out of 14 samples**.** Taking commercially produced Neem Oil (L1, L2) into consideration; average value of 16 samples is worked out to be 2868 ppm Nimbin. All these data indicate that it could be possible to achieve **3000 ppm Salannin** on an average during seasonal Neem oil recovery on a large scale.

Study of other vegetable oils: Table 7 gives data of important characteristics for other oils, which are likely to be blended with Neem Oil by suppliers to increase their profit margins. Neem limonoids like Azadirachtin, Nimbin and Salannin are absent in these oils. Blending of any of such oils will dilute the limonoid concentration of Neem oil. By fixing minimum limits of these limonoids in Neem Oil specifications, it would be possible to exercise effective quality control over quality of Neem oil in indirect way.

Further, **Mineral oil has 98.7% unsaponifiable** matter, whereas vegetable oils are having it in the range of 2-3 %. By finding out proportion of unsaponifiable matter in Neem oil, it would be possible to judge Neem oil's blending with Mineral Oil.

Iodine value and saponification no. of other vegetable oils do not show drastic difference in comparison to Neem Oil. Therefore, these parameters cannot reveal part blending of neem oil with any of these oils.

Table 7: Analysis data of other oils which are likely to be blended with Neem oil by suppliers.

Oil	Acid value	Sap number	Iodine value	Un-sap matter	Aza	Nimbin	Salannin
	mg KOH /gm	*mg KOH /gm*	*mg I2 /gm*	*%*	*ppm*	*ppm*	*ppm*
Cotton seed oil	0.6	193.4	102.2	1.69	ND	ND	ND
Rice bran oil	2.4	180.3	114.4	4.9	ND	ND	ND
Transformer oil (Mineral)	0.7	ND	ND	98.74	ND	ND	ND
Karanj oil	11.5	186.3	95.5	0.43	ND	ND	ND
Palm oil	0.98	190.82	59.07	1.12	ND	ND	ND

Study of Azadirachtin rich materials: Because of similarity of Neem Oil properties with many other vegetable oils, Azadirachtin level is presently used as a quality control criteria of Neem oil. To supply more neem oil quantity than production capacity, neem oil is mixed with other spurious oils having similar characteristics and Azadirachtin rich materials to meet with specifications. Therefore, Technical Grade Azadirachtin & Neem Based Insecticide was evaluated for Azadirachtin, Nimbin and Salannin concentrations, as shown in Table 8.

The data reveals that these sources are rich in Azadirachtin, but they possess lesser concentrations of Nimbin & Salannin. Mixing of such materials can raise the level of Azadirachtin in economical way. However, it would be highly uneconomical to meet with criteria of Nimbin & Salannin by mixing these materials. Therefore, incorporation of Nimbin and Salannin criteria as described above in Neem oil specifications could act as a deterrent for mixing of other oils & Azadirachtin rich materials in Neem Oil.

Table 8: Concentration of Nimbin & Salannin in Azadirachtin enriched materials.

Azadirachtin source	Sample code	Aza	Nimbin	Salannin
		ppm	*ppm*	*ppm*
Azadirachtin Tech - 35% pure powder	T1	35%	0.29%	0.86%
Azadirachtin Tech - 4% solution in n-butanol	T2	40600	ND	ND
Neem Based Insecticide containing 1% Azadirachtin	I1	9140	2395	7101
Neem Based Insecticide containing 5% Azadirachtin	I2	50000	301	619

CONCLUSION

Purity of Neem oil being used for producing Neem Coated Urea is being primarily determined on the basis of several physic- chemical characteristics and Azadirachtin content of 150 ppm minimum. There is a strong possibility that most of the limits are complied with by adultering neem oil with other vegetable or mineral oil and mixing of Azadirachtin powder to meet with limit of 150 ppm Azadirachtin. Therefore the present criteria seem to be inadequate and technically insufficient.

The intrinsic quality of neem oil is due to Azadirachtin along with other active ingredients like Nimbin and Salannin. On the basis of this study, it becomes evident that there is a strong need to incorporate limits of other active ingredients of Neem like Nimbin and Salannin as governing criteria for ascertaining neem oil quality. Limits of Nimbin & Salannin are recommended at the levels of 2500 ppm and 3000 ppm respectively, as it is seen that seasonal extraction activity of neem fruits are giving better extraction of Neem's active components to meet with recommended levels. The study of pure neem oils from neem seeds of different geographical locations have also shown that Azadirachtin level can be 75 ppm or more, which implies that there is a need to reduce limit of Azadirachtin content from 150 ppm to 75 ppm. This revision in specification will not change neem oil's basic properties. By introducing composite specifications of Azadirachtin, Nimbin and Salannin, it will be possible to judge that adulteration of Neem Oil has taken place, even if it is topped up with Azadirachtin powder.

There is also a scope to narrow down the limits of saponification value and iodine value prescribed in FCO. Also, the incorporation of limit for unsaponifiable matter will prevent mixing of mineral (transformer or cutting) oil into Neem Oil.

In a nut shell, this study indicates that following criteria of neem oil would ensure proper quality control of neem oil used for producing Neem Coated Urea:

1 Moisture & insoluble impurities, % by mass: 1.0 max
2 Specific gravity at 30 deg C: 0.85 to 0.95
3 Iodine value: 68 to 90
4 Saponification value: 170 to 190
5 Unsaponifiable matter: 4% max
6 Azadirachtin content, ppm: 75 minimum
7 Nimbin content, ppm: 2500 minimum
8 Salannin content, ppm: 3000 minimum

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