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# BIOPESTICIDES AND NEUTRACEUTICALS FROM PLANTS

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**ABSTRACT**:

Bio-pesticides gained lot of importance because of eco-friendly nature, keeping the environment clean and green and also less toxic compared to synthetic pesticides. The most important bio-pesticides prepared from the extracts of plants are neem, custard apple, karanja, mahua and Asoka etc. Some of the aspects relating to the process for the preparation of bio-pesticide from the said plants, isolation and identification of active compound, enrichment of active compound from extracts and analytical method for the quantification of active compound in the technical material and also in formulations will be presented. Process for the preparation of nutraceutical lycopene enriched oleoresin from tomato fruit and its estimation by HPLC will be presented.

**KEY WORDS:** *Biopesticides, Neutraceuticals, Plants.*

**INTRODUCTION:**

Higher plants constitute a treasure house of biologically active compounds which can be developed as safe bio-pesticides for crop protection from variety of pests. The selection of plants species for investigation is usually based on their reported uses in the traditional systems. Knowledge of toxic plants and their biological activity is a paramount importance, not only to utilize them as a natural insect control agent and replace the toxic commercial chemical insecticides but also to understand the nature of their toxicity on non toxic species.

One of the approaches in recent years has been the use of clean chemistry or what is termed as Green Chemistry. This type of chemistry implies the design, development and implementation of chemical products and processes that would eliminate the use of hazardous substances to human health and environment (Koul, 2002). Pesticides derived from plant sources have emerged as important eco safe alternatives in the management of pests and diseases in agriculture during the last decade though their adoption by farmer is still infancy.

Pesticides derived from plant sources are selectively toxic do not bio accumulate and relatively short persistence in the environment and are more wanted in the modern integrated pest management programmes (IPM). Plant derived insecticides are ecofriendly because they have been found to be selective (Saxena et al, 1984) and pose less negative impacts to ecosystems than conventional insecticides (Stark, 1992). Interest in plant based pesticides has grown over the last two decades because several synthetic pesticides are eliminated from use due to environmental and food safety problems (Koul et al., 1990; Prakash and Rao, 1997; Kumar and Singh, 2002; Dhaliwal and Koul, 2007). The plant kingdom provides a rich source of chemicals possessing diverse biological activities on insects. Several plants possessing pest control properties have been identified (Koul et al., 1990; Grainge and Ahmad, 1988; Nelson and Venugoplal, 2006 and Dhaliwal and Koul, 2007).

The use of synthetic pesticides to control the insects has posted substantial problems due to their persistence in the environment, insect resistance and high mammalian toxicity. The integrated pest management (IPM) technique is one of the new approaches where adaptation of different suitable measures in an integrated manner can manage the pest population at a safe level without causing economical injury. The eco-friendly pesticides may be defined as one which is less harmful to environment and human health. The use of these pesticidal molecules will be suitable in controlling pest population without affecting eco-system (Roy, 1998). On the other hand, plant derived insecticides are more readily biodegradable, less likely to contaminate the environment and less toxic to insect pests.

The most important and well known plant based pesticide is *Azadirachta indica* (neem) having diverse biological activities. Earlier we developed a process for the preparation of technical material (H.C. Sharma et al, 1984; A.V.B.Sankaram et al, 1999) odour less pale yellow oil and meal free of neem smell (A.V.B.Sankaram et al, 1987) from neem kernels. Pesticides prepared from *Annona squamosa* (custard apple) (AVB Sankaram et al, 2006 and *Pongamia glabra* (Karanja) (M M Murthy et al, 2010) are also effective in controlling different pests of agricultural. In this paper the preparation of biopesticides from the seeds of these plants, isolation and enrichment of active compound and its quantification will be presented.

**MATERIALS AND METHODS:**

Preparation of biopesticide from the seeds of neem, custard apple and karanja:

The seeds of these plants, neem, custard apple and Karanja are collected in Hyderabad during May- July for neem, October-November for custard apple and Jan- March for karanja. The shade dried seeds were powdered in a domestic mixer to a particle size ranging from 0.2 mm to 2.5 mm. The seed powder was extracted with polar solvents like alcohols methanol or ethanol etc at ambient temperatures. The polar extract was concentrated under reduced pressure or at ambient temperature and the concentrated extract was fractionated with non-polar solvent. The insoluble of non-polar solvent was further treated with water and water immiscible organic solvents like ethyl acetate, chloroform, dichloromethane and ether. The organic phase was separated concentrated and the semi-solid residue is technical material which is called biopesticide (chart-1). The technical material so obtained was formulated which ultimately goes to agricultural fields. The bio pesticide can also be prepared from expelled neem and Karanja oil as shown in the chart -2.

The active compound was isolated from the technical material by column chromatographic techniques and finally by HPLC method. Azdirachtin A, Squamocin, Karanjin and lycopene (Figure-1) are the active compounds present in the extracts of neem ,custard apple, karanja and tomato respectively. The active compound is also enriched from the crude extracts by solvent fractionation.

The residual seed powder after polar solvent extraction is further extracted with non-polar solvent to get oil. The by-products obtained in this process are oil and cake as shown in the chart -3.

The estimation of the active compounds is performed by analytical HPLC method and conditions are shown in chart – 4

**RESULTS AND DISCUSSIONS:**

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The residual seed powder after polar extraction is further extracted with non-polar solvent to get oil. The by products are oil and cake as shown in chart -2. The estimation of the active compounds is performed by analytical HPLC method and conditions are shown in chart - 3

Lycopene an acylic carotenoid, abundantly available in tomatoes (Lycopersicon Esculentum) is mainly responsible for the characteristic red colour in tomato fruits. Lycopene is not merely a pigment. Lycopene is also a powerful antioxidant that can protect against prostate cancer, breast cancer, and atherosclerosis and associated coronary artery diseases. Lycopene also reduces LDL (Low density Lipoprotein) oxidation and helps to reduce cholesterol level in the blood. Lycopene is a powerful nutrient in the human dietary system. Tomatoes are the second largest crop in India.

Extraction method for the preparation of lycopene enriched oleoresin has been developed on a batch size of 5 kg of tomatoes. The method involves crushing of tomatoes and separating the aqueous part (97.6%) to obtain semidried pulp (8-12%) which is then treated with mixture of organic solvents followed by filtration to get the crude lycopene solution. The so obtained solution is further treated with water to remove the water soluble components and concentrate under vacuum to get crude oleoresin (yield 0.062%) containing lycopene. 15 batches of such extractions were carried out and obtained crude oleoresin 50gms containing about 3.2% of Lycopene. This oleoresin was further purified by preparative thin layer chromatography (PTLC) yielding lycopene of purity 92%. Simple qualitative analytical HPLC method has been developed. (Chart-5)

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**REFERENCES:**

Dhaliwal, G.S.and Koul, O. (**2007**). Biopesticides and Pest Management: Conventional and Biotechnological approaches. Kalyani Publishers, New Delhi, 455 pp.

Grainge, M. and Ahmad, S. (**1988**) Hand Book of Plants with Pest Control Properties. John Wiley & Sons, New York.

Koul, O (2002). Let us think green and make chemical pollution prevention our motto, save the environment and ensure safety of human habitat, Biopesticide News, 6, august 2002, Vol-6, 2, pp 2.

Koul, O., Isman, M.B. and Ketkar, C.M. (**1990**). Properties and uses of neem, *Azadirachta indica* A.Juss. *Can.J. Bot.,* 68, 1-11

Kumar, M. and Singh, R. (**2002**). Potential of *Pongamia glabra* vent as an insectide of plant origin. *Biol.Agri.Hort,* 20, 29-50.

Murthy, M M., et al, (**2010**). India and PT556, NF- 65/09, 2010/DEL/09 Process for the isolation of karanjin from expelled and solvent extracted cake of *Pongamia glabra*

Nelson, S.J. and Venugoplal, M.S. (**2006**). Antifeedant and growth disruptive effects of various plant products on *Spodoptera litura F*. (Lepidoptera: Noontide). *J.Ent.Res.* 30, 93-102.

Prakash, A. and Rao, J (**1997**). Botanical Pesticides in Agriculture; Boca Raton: CRC Press FL 480 pp.

Roy N K and Dureja, P (1998). New friendly pesticides for Integrated Pest Management, Pesticides World, April-May 1998, pp 16-22.

Saxena. R.C.,Justo,H.D.,JR. and Epino,P.B. (**1984**). Evaluation and utilization of neem cake against the brown plant hopper, Nilaparvata lugens (Homoptera: Delphacidae). *J.Econ.Entomol*. 77,502-507.

Stark, J.D (**1992**). Comparison of the impact of a neem seed kernel extract formulation, Margosan-O and Chloropyriphos on non-target invertebrates inhabiting turf grass. Pestic.Sci. 36 293-299

Sharma, H.C., Leuschner, K. Sankaram, A.V.B., Gunasekhar, D., Marthandamurthi, M., Bhaskaraiah, K., Subramanyam, M. and Sultana, N. (**1984**). Insect antifeedants and growth inhibitors from *Azadirachta indica* and *Plumbago zeylanica*, "Natural pesticides from the neem tree and other tropical plants", proceedings of the 2nd International Neem Conference, Ed, H. Schmutterer and K.R.S. Ascher (Rauisschholzhausen FRG, 25-28, May 1983). Dt. Fur Techn. Zusammenarbeit (GTZ), Eachbrorn, FRG, p. 291,

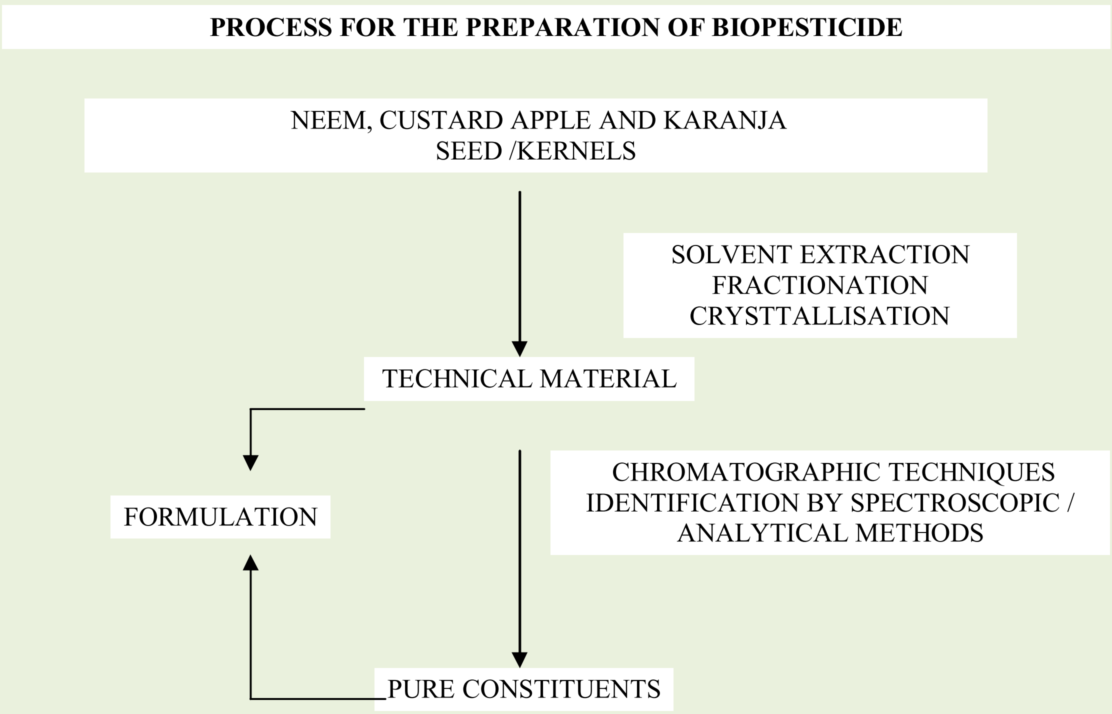
Sankaram, A.V.B., Marthandamurthi, M , Bhaskaraiah, K., Subramanyam, M., Sultana, N., Sharma, H.C., Leuschner, K., Ramprasad, G., Sitaramaiah, S., Rukmini C.and Udauasekhara Rao, P. (**1987**). Chemistry, biological activity and utilization aspects of some promising Neem extractives, "Natural Pesticides from the Neem tree and other tropical plants", proceedings of the 3rd International Neem Conference, Ed. H. Schmutterer and K.R.S. Ascher (Nairobi, Kenya 10-15, July, 1986), Dt. Ges. Fer. Techn. Zusammenarbeit (gtz), Eschborn, FRG, P. 127

Sankaram A.V.B. et al (**2006**). US Patent No. 6,991,818 Jan.31 .

Compound Iso-squamocin obtained from seeds of *Annona squamosa* composition containing the same

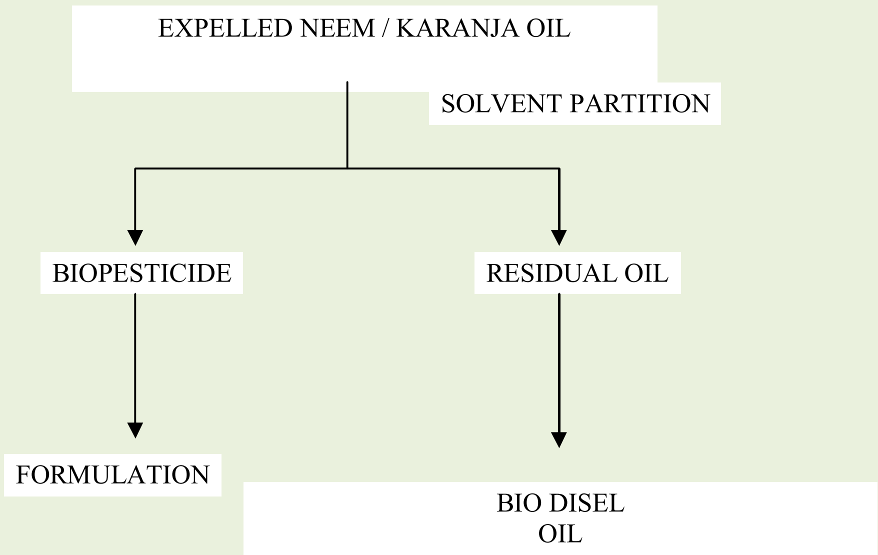
Sankaram A.V.B. *et al* (**1999**).Pesticidal dry powder formulation enriched in azadirachtin upto 88%. An emulsifiable concentrate enriched upto 30% of azadirachtin and a process for preparing such formulation and concentrate from neem seed/kernel.

## CHART-1

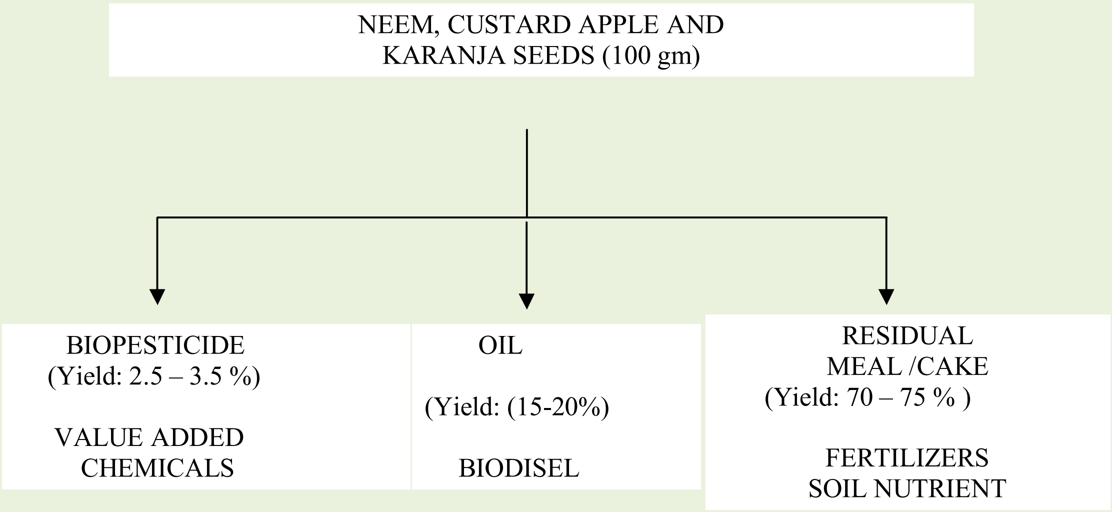



## CHART-2

DEVELOPMENT OF PROCESS FOR THE PREPARATION OF BIOPESTICIDE FROM NEEM OIL / KARANJA OIL



## CHART-3




## CHART- 4

HPLC CONDITIONS FOR THE ESTIMATION OF ACTIVE COMPOUND IN NEEM, CUSTARD APPLE AND KARANJA EXTRACTS

| ANALYTICAL | NEEM | CUSTARD APPLE | KARANJA |
|---|---|---|---|
| STATIONARY PHASE | C-8, µBONDAPAK (150MMX4.5MM) | C-18, µBONDAPAK (150MMX3.9MM) | C-18, µBONDAPAK (150MMX3.9MM) |
| MOBILE PHASE | $CH_3CN : H_2O$ (26:74) | MEOH: H2O (82:18) | MEOH :$H_2O$ (85: 15) |
| WAVELENGTH | 215 NM | 220 NM | 305 NM |
| DETECTOR | UV | UV | UV |
| FLOWRATE | 0.8 ML/MIN. | 0.6 ML /MIN. | 0.5 ML/ MIN. |

**CHART-5**

HPLC CONDITIONS FOR QUANTIFICATION LYCOPENE:

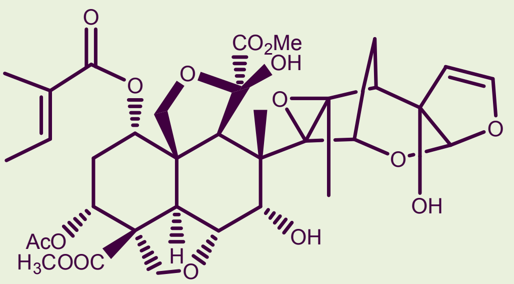
STATIONARY PHASE: C-18 SUPELCOSIL LC 18(15 CM X4.6 CM, 3 MM)

MOBILE PHASE: $CH_3CN$: MEOH: THF (70:20:5)

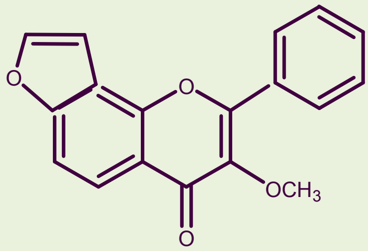
FLOW RATE: 1 ML/MINUTE

DETECTOR: 475 NM

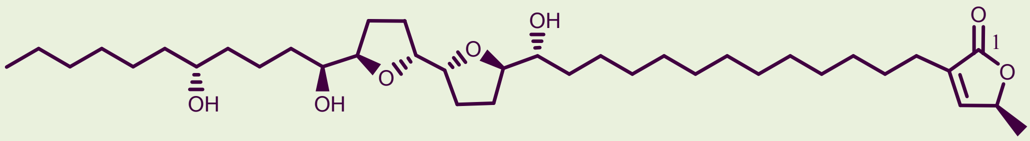
LYCOPENE RT: 14.30 MTS



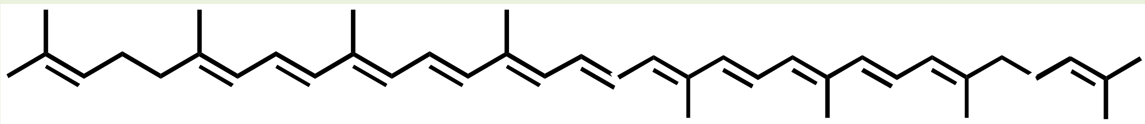
**Azadirachtin A**



**Karanjin**



**Squamocin**



**Lycopene**

**(Figure-1)**