

Preliminary report on use of Neem and Negundo leaves in preservation of library and archival materials

Presented by

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**use of Neem and Negundo
leaves in preservation of
library and archival materials**

Introduction

- Use of Neem and Negundo leaves in preservation of materials is a traditional practice in India
- These are natural insect repellents have been in use for many years
- Have proved effective against cockroaches, silverfish and book lice and fungal attack.
- Insect repellent property is due to presence of presence of active phytochemicals in the leaves, which give characteristic essence to the leaves

Use of essential oils as insect repellents

- Insects plays a major role in deterioration of objects
- Use of pesticides and insecticides is one of the means of preventing losses from insects
- The search for active natural products derived from plants as ecologically safe alternation
- globally there is growing awareness and desire to utilize natural and environment friendly compounds for pest control
- In this connection essential oils have been shown to possess a broad spectrum of pest control properties

Essential oils have been widely investigated for their activities like,

- ✿ Larvicidal
- ✿ Toxic
- ✿ Repellent
- ✿ Ovicidal
- ✿ Antifeedant
- ✿ Antioviposition

As part of screening programme for natural phytochemicals, active botanicals that grow in India were chosen

- ◆ *Azadirachta indica* (Neem, margosa tree)
- ◆ *Vitex negundo linn* (Nirgandi)

Azadirachta indica (Neem)



- ❑ one of the most versatile medicinal plant having a wide spectrum of biological activity
- ❑ Every part of the tree has been used as traditional medicine
- ❑ The Sanskrit name of the Neem tree is 'Arishtha' meaning 'reliever of sickness' and hence is considered as 'Sarbaroganibarini'
- ❑ The tree is still regarded as 'village dispensary' in India



Chemistry of neem leaves

Neem leaf is a reservoir for vast number of active natural compounds, which is attracting researchers throughout the world.

Neem leaf mainly yield volatile fractions containing sesquiterpene, diterpenes, tetranortriterpenes as well as number of limonoids like nimbin and its derivatives, nimocinolide, isonimocinolide

Fresh matured leaves yield an odorous viscous essential oil which contains complex mixture of volatile compounds

Some bioactive compounds from neem leaves

Neem compound	Biological activity	Refernce
Nimbidin, Sodium nimbidate, Nimbin, Nimbolide	Anti-inflammatory, Antiarthritic, Antipyretic, Hypoglycaemic, Antigastric ulcer, Spermicidal, Antifungal, Antibacterial, Diuretic	Pramila thakkar, neem foundation www.neemfoundation.org
Meliatetraolenone , odoratone	Antifungal, Antimalarial, insecticidal Antimalarial	BS Siddiqui et al. 2003
Zafaral, meliacinanhydride	Antifungal, Antimalarial, insecticidal Antimalarial	BS Siddiqui et al. 2003
6 α -O-acetyl-7-deacetylnimocinol , meliacinol	Antibacterial , Antimalarial	BS Siddiqui et al 2000
Nimonol, nimonolactone, nimonolide	Anti-inflammatory and antifeedant	Geetha Gopalakrishnan et al. 2002
isomeldenin	Antifungal	G Suresh et al. 1997
Kaempferol-3-O-rutinoside Kaempferol-3-O- β -D-glucosid	Antioxidant	Chattopadhyay RR et al. 2005

Vitex negundo (Nirgandi)

■ Botanic description

Vitex negundo is a much branched shrub with quadrangular, densely whitish branchlets, up to 5 m tall or sometimes a small, slender tree. thin grey Bark, slightly greyish green Leaves. Flowers are bluish-purple and it is widely planted as a hedge plant along roads and between fields.

Found throughout the greater part of India, often occurring gregariously; it is abundant along river banks, in moist situations, open wastelands and near deciduous forests. Ascending to an altitude of 1 500 m in the outer Himalayas.

Negundo



Botanical name – Vitex negundo Linn

Common names

- (English) : five-leaved chaste tree
(Hindi) : Nisinda, sambhalu, Nirgandi
(Kannada) : Niragundi, Lakkigida, Nekka
Nekkilu
(Malayalam) : Karonocci
(Tamil) : Nirkundi, Nallanocci
(Telugu) : Nallavavili, Vavili, Tellvavili







biological properties of vitex negundo

- All parts of the plant are commonly used in Indian medicine
- Leaves have insecticidal properties and are laid over stored grain to ward off insects
- They show anti-inflammatory, antibacterial and anti-fungal activity (Banerjee AK, 1989 and Tandon VR, 2005)
- plant oils were effective in checking insect infestation. (A. Rahman *et al*)
- lipophilic extracts of Vitex negundo shown antifungal activity (Sanjay Guleria *et al* 2006)

Chemical constituents of volatile fractions of negundo leaves

Volatile fractions extracted from negundo leaves contains many compounds, namely the major components of *V. negundo* L. from India, China and Philippines were β -caryophyllene, viridiflorol and β -eudesmol (5).

- viridiflorol;
 - squalene;
 - β -sitosterol;
 - 5-hydroxy-3,6,7,3',4'-pentamethoxy flavone;
 - 5-hydroxy-3,7,3',4'-tetramethoxy flavone;
 - 5, 3'-dihydroxy-7,8,4'-trimethoxy flavanone;
 - *p*-hydroxybenzoic acid;
 - 3,4-dihydroxy benzoic acid;
 - luteolin 7-glucoside; isoorientin;
 - agnuside and
 - 2'-*p*-hydroxybenzoyl mussaenosidic acid
- were isolated and characterized by spectral data (UV, IR, NMR & MS) from the different extractives of the leaves. (ref Virendra singh et al.1999 & HadjMohammadi et al. 2006)



Extraction of volatile oil fractions from Neem and Negundo leaves

Volatile essential oil was extracted from leaves by hydrodistillation of air dried leaves using Clevenger apparatus

- About 1 kg of air dried leaves were taken in a round bottom flask and water is added, distilled continuously for 4 to 5hr at a low temperature,
- volatile compounds which have very boiling points gets collected in the side tube,
- Around 0.5-1mL of liquid fractions were collected in a stoppered bottle
- Oil fractions were collected in a non volatile solvent to lower their volatility and stored at 10-15°C



Extraction of volatile essential oil fractions using Clevenger apparatus

Volatile oil fractions extracted from Neem and negundo leaves

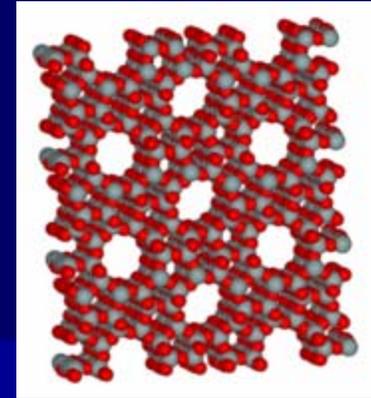


Preparation of mats

Materials used:

- ◆ Hand made paper
- ◆ Zeolite
- ◆ Sodium stearate
- ◆ Sodium lauryl sulphate

Material chemistry



- Zeolite

It is a sodium aluminium Silicate mineral found abundantly, available in different forms . It is mainly used as adsorbent Material for volatile compounds.

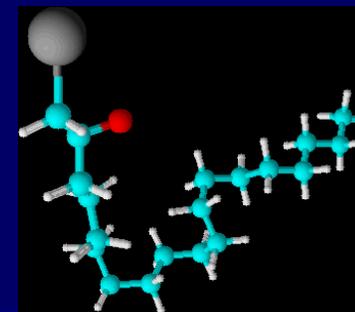
Type used Zeolite Nax/fau

Supplied by: Ranbaxy fine chemicals limited,

New Delhi-110020



Sodium stearate



It is a sodium salt of stearic acid . It is commonly called as soap. It is used as surfactant and It is used to trap oily substances

Supplied by:

S.V. Enterprises,

Mumbai-400009

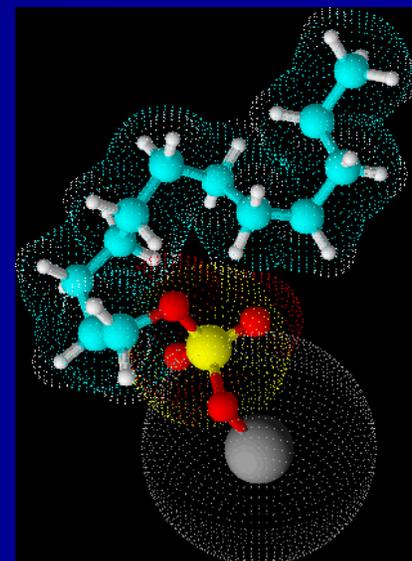
Sodium lauryl sulphate

It is a anionic detergent. It is used as surfactant , it is used to trap oily and greasy materials. It is a common constituent of synthetic detergents and washing powders

Supplied by:

NICE Chemicals Pvt. Ltd.

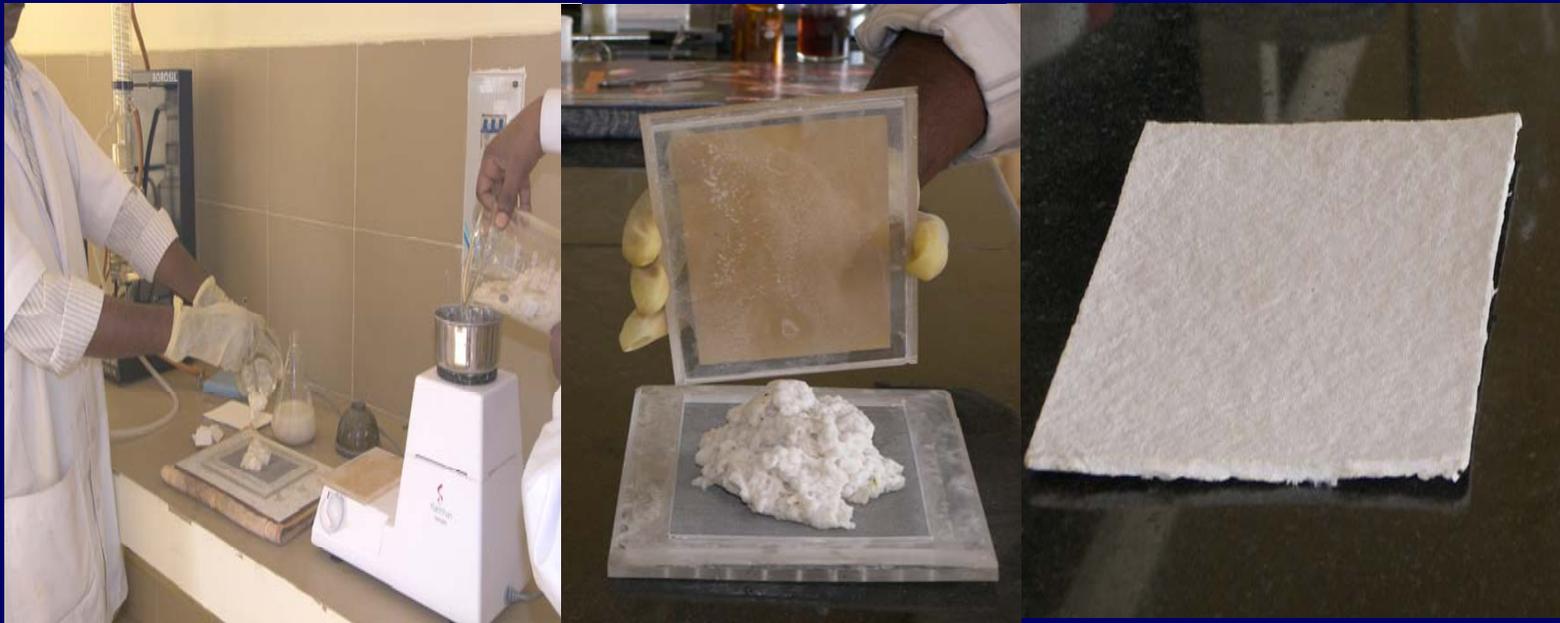
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Method of mat preparation

- Mats similar to commercially available mats were prepared in our laboratory, it involves following steps
- Hand made paper was taken ,cut into small pieces soaked overnight in water ,from this slurry of paper was prepared
- Paper pulp was mixed with definite proportion of fixing agents like Zeolite, sodium Stearate, Sodiumlaurylsulphate
- Poured into a mould, cast into a sheet, dried at room temperature .Cut into small pieces of size 3×2cm.
- Mats were impregnated with volatile oily fractions by dipping in solution of oils at least 3 times, finally dried at RT kept in desiccators.

Process of mat preparation



Testing of mats

- Aroma retaining duration
- Antifungal assay of volatile aroma

Testing of mats for their aroma retaining duration

Testing of mats containing aroma volatile was done by keeping them in closed chamber made of Perspex sheet (PMMA),

- At RT and
- Electric mat heater

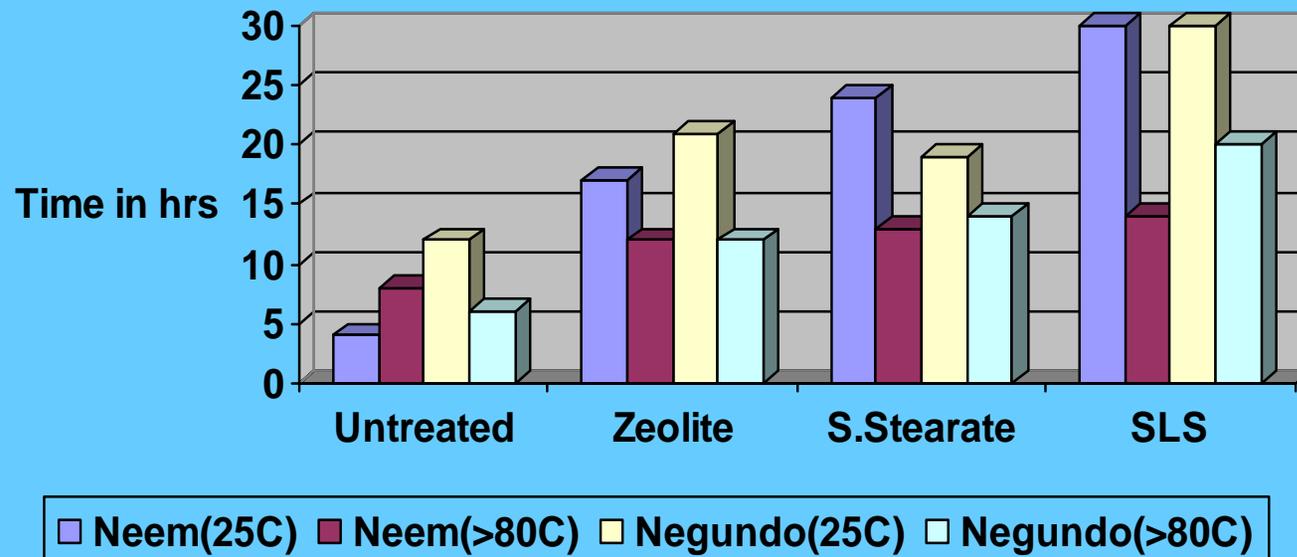


Results and discussion

Aroma source	<i>Azadirachta indica A juss</i>			
Aroma Fixing agent used	Untreated	Zeolite	Sodium stearate	Sodium lauryl sulphate
At room temperature, 25.C	4 hrs	17 hrs	24 hrs	30 hrs
When heated in a electric mat heater	8 hrs	12 hrs	13 hrs	14 hrs

Aroma source	<i>Vitex negundo</i>			
Aroma Fixing agent used	Untreated	Zeolite	Sodium stearate	Sodium lauryl sulphate
At room temperature ,25.C	12 hrs	21 hrs	19 hrs	30 hrs
When heated in a electric mat heater	6 hrs	12 hrs	14 hrs	20 hrs

Aroma retaining duration of mats



- Mats prepared by impregnating Neem and negundo oil extracts have shown aroma retaining property
- It is clear from the graph ,i.e. aroma retained for longer duration by treated mats compared to untreated mat and aroma was quickly released when heated in electric mat heater



Neem (*A. indica*) and Negundo (*V. negundo*)

Bioassay

- ❖ Antifungal activity of volatile components i.e., essential oils of Neem (*A. indica*) and negundo (*V. negundo*).
- ❖ Insect- repellent activity of mats impregnated with essential oils .
- ❖ Insecticidal activity of mats impregnated with essential oils .

Antifungal activity of volatile components i.e., essential oils of Neem (*A. indica*) and negundo (*V. negundo*)

Test method

- ❖ Fungal spore suspension was prepared using grown fungal cultures.
- ❖ The antifungal activity of volatile components of neem and negundo leaves extracts were assessed using microtiter plate, wells filled with fungal spore suspensions. (*Wilson, C.L., Solar, J.M., Ghaouth, A.El., Wisniewski, M.E., (1997)*)
- ❖ After inoculation of test strains, to test volatile fungicidal activity of oils, 50 µl was placed on small 8 mm disk containing adsorbents/carriers. Individual microtiter wells were then covered with paper disc.
- ❖ The fungal inhibition was observed by inoculating suspension on (SDA) Saboraud dextrose agar medium.

Assessment of volatile component as antifungal....



Picture: Inoculation of microtiter wells with fungal spore suspension



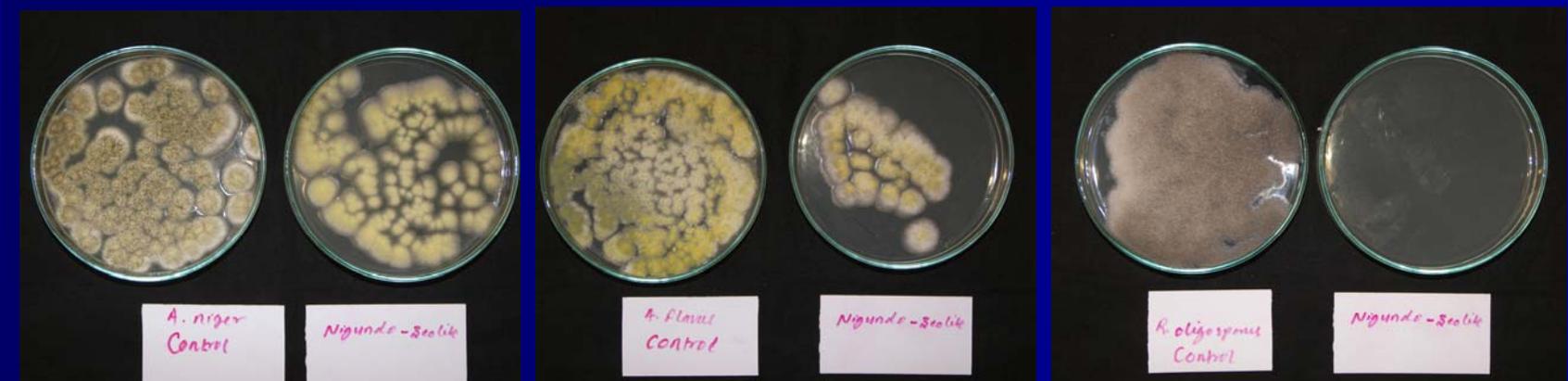
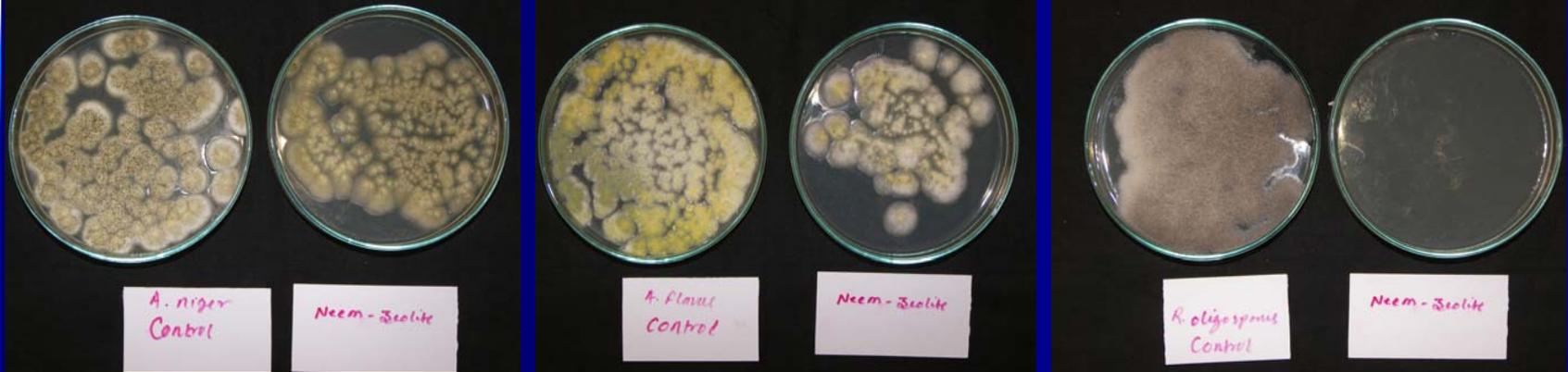
Mats impregnated with essential oils.

Assessment of volatile component as antifungal...



Testing of mats as antifungal...

Antifungal activity of volatile components i.e., essential oils of Neem (*A. indica*) and negundo (*V. negundo*)



Antifungal activity of volatile components i.e., essential oils of Neem (*A. indica*) and negundo (*V. negundo*)

S. No.	Mats	Fungi		
		<i>A.niger</i>	<i>A.flavus</i>	<i>R. oligosporus</i>
1.	Neem - SLS	+	++	+++
2.	Neem - Zeolite	+++	+++	+++
3.	Neem - S. stearate	+++	++	+++
4.	Negundo - SLS	++	++	+++
5.	Negundo - Zeolite	+++	+++	+++
6.	Negundo - S. stearate	++	++	++

- + indicate less growth as compare to control
- ++ indicate relatively less growth
- +++ very less/no growth

Antifungal activity of volatile components i.e., essential oils of Neem (*A. indica*) and negundo (*V. negundo*)

Evaluation of volatile components as fungicidal...

- ❖ In comparison to control, growth was classified in to three classes
- ❖ With the use of all mats growth was inhibited completely in case of *R. oligosporus*
- ❖ Among the mats tested after analysis neem and negundo mats in combination with zeolite as carrier was found to good among all.

Insect repellent activity of volatile components i.e., essential oils of Neem (*A. indica*) and negundo (*V. negundo*)

Picture. Silverfishes

Silverfish (*Lepisma saccharina*), primitive wingless insects, feed on a variety of materials, including paper, cotton, starch, and cereals.



They can be a problem in libraries and other places where books, documents, and papers are stored used as test organisms for the assay.

Insect- repellent activity of mats impregnated with essential oils.

- ❖ The test chamber of size 3 cubic sq ft equipped with electric mat heater was used to analyze insect repellent activity by analyzing and observing its movement in presence and absence of mats. *Sheng-Yang Wang and et al.,(2006),*
- ❖ The insect repellent activity of paper mats impregnated with essential oils, was tested by observation of movement of silver-fishes (*Lepisma saccharina*) in response to fumigation by heating mats.

Insect- repellent activity of mats impregnated with essential oils.

Observation in presence of mat

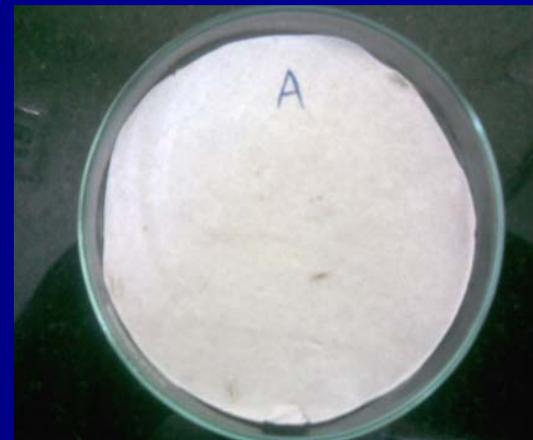
The movement observed in presence of mat impregnated with extracts, both the extracts found to be effective as very rapid movement of silverfishes was observed.

To assess silverfish mortality by application of mats impregnated with essential oils.

Insectidal test

- ❖ The closed test chambers made with transparent Perspex sheets were used for testing mortality of selected insect.
- ❖ The chamber was equipped with electric mat heater. Insects were placed in Petri plates without lid was then placed in chamber and observed after each 10 min, until each insect get killed. *Sheng-Yang Wang and et al.,(2006)*
- ❖ The time was noted against mortality.

To assess silverfish mortality by application of mats impregnated with essential oils.



To assess silverfish mortality by application of mats impregnated with essential oils.



Picture: analysis of insect mortality

To assess silverfish mortality by application of mats impregnated with essential oils.

Set No	Control			
	1	2	3	4
1	-	-	-	-
2	-	-	-	-
3	-	-	-	-
4	-	-	-	-

Set No	Neem mats			
	1	2	3	4
1	+	+	+	+
2	+	+	+	+
3	+	+	+	+
4	+	+	+	+

* - indicate survival of silverfish

* + indicate killing of silverfish

To assess silverfish mortality by application of mats impregnated with essential oils.

Set No	Control			
	1	2	3	4
1	-	-	-	-
2	-	-	-	-
3	-	-	-	-
4	-	-	-	-

Set No	Nigundo mats			
	1	2	3	4
1	+	+	+	+
2	+	+	+	+
3	+	+	+	+
4	+	+	+	+

* - indicate survival of silverfish

* + indicate killing of silverfish

To assess silverfish mortality by application of mats impregnated with essential oils.

Observations

- ❖ The results for mortality was very significant as 100% mortality was achieved using both test sample.
- ❖ It was observed that about 1 ½ hr was sufficient for killing of all larvae in the case of neem mats.
- ❖ In case of negundo mats all larvae were killed in relatively less time in about 1 hr.

Thank you.....