Detection Of Biosal (Neem Formulaion®) By HPLC After 24 Hours Treatment In Callosobruchus Analis

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ABSTRACT
Toxicity of Biosal essential oil against *Callosobruchus analis* by three methods Direct Application method (DAM), Glass film method (GFM) and Filter paper impregnation method (FIM) after 24 hours of treatment. The LC_{50} value calculated as 1.338504 µl/cm^2 by DAM, 12.174 µl/cm^2 by GFM and 6.945603 µl/cm^2 by FIM. HPLC analysis showed the activity of Biosal against *C.analis* by (DAM) represented by peak area in µm. The seed extract Azadirachtin (standard) shown by one peak 102387, peak (2) and (3) were absent and in Biosal sprayable (sample) shown by two peaks 111990, 2679, peak (3) was absent, while by (GFM) Biosal sprayable (standard) shown by four peaks showed as 6743, 62559, 6765, 1842 µm and in Biosal sprayable (sample) shown by four peaks which was 10818, 37728, 2954, 1277 µm, similarly by (FIM) Azadirachtin (standard) shown one peak 1339, peak 61568 and (3) was absent and Biosal sprayable (sample) shown as 7527, 30040, 1013 µm.

Key words: HPLC High performance liquid chromatography, Biosal Neem Phytopesticides, Callosobruchus analis stored grain pests treated by phytopesticides.

INTRODUCTION

Koul *et al (*1990) and Schmutterer (1990) worked on neem, *Azadirachta indica*, properties and its uses. Hull *et al* (1993) reported Quantitation of azadirachtins in pesticidal formulations by high-performance liquid chromatography. Naumann (1994) reported Systemic action of neem seed extracton mountain pine beetle (Coleoptera: Scolytidae) in lodgepole pine. Schmutterer and Singh (1995) reported the List of insect pests susceptible to neem products. In: Schmutterer H.(ed.): The Neem Tree *Azadirachta indica* Zounos (1999) reported Bioactive compounds from neem tissue cultures and screening against insects. Naumann (1996) worked onToxicity of a neem (*Azadirachtaindica* A. Juss) pesticides to larvae honey bees. Johnson and Morgan (1997) worked onNeem (*Azadirachta indica*) seeds. Comparasion of chromatographic system for triterpenoid Pavela and Holy (2003) reported the Effects larvae of *Mamestra brassicae*. *Spodoptera littoralis Lymantria dispar*, and Horticulture and Vegetable Growing by using insecticide azadirachtion Podhorniak *et al* (2004) reported the multiresidue method for N-methyl carbamates and metabolitepesticide residues at the parts per billion level in selected representative commodities of fruit and vegetable crop groups.Koul *et al* (2004) worked on the lepidopteran larvae using activity of some non azadirachtin limonoids from *Azadirachta indica* insecticides Koul *et al* (2004) worked

on the activity of some non azadirachtin limonoids from *Azadirachta indica* against lepidopteran larvae. Silva *et al* (2007) Purification of the seven tetranortriterpenoids in neem (Azadirachta indica) seed by counter-current chromatography sequentially followed by isicratic preparative reserved-phase-high performance liquid chromatography Sarais *et al* (2008) reported a simple and selective method for the measurement of Azadirachtin and Related Azadirachtoid levels in Fruits and vegetable using liquid chromatography Electrospray ionization tendm Mass spectrometry.Coventry and Allan (2010) determined the microbiological and chemical analysiss of Neem (*Azadirachta indica*) Extract:

MATERIAL AND METHODS:

QUANTITATIVE ANALYSIS OF PESTICIDE RESIDUES BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC):

During the recent years chromatography technique have undergone a rapid development and high performance liquid chromatography (HPLC) is one the achievement. HPLC have been used for the separation of pesticides, by using a packed column (Zorbax TMNH2) a polar bounded phase with particle size of about 7 HM in diameter. Standard sample of Neem as well as the samples from animal tissues (strain of *Callosobruchus analis* treated with Neem pesticides). The column was used with fractionated n-hexane as mobile phase with a flow rate of 2.5 ml/min. A UV detector was used with a wavelength of 205 nm, pressure 200 kg/cm^2 and the absorbance 0.08 with chart speed 10 mm/ min for detection of pesticide. The columns are packed to uniform bed density by using a high pressure slurry loading technique. The column was used with fractionated n-hexane as mobile phase with a flow rate of 2.5 ml/min. Initial separation of interfering organic residue such as fats, pigments and other organics must be carried out. Extraction of fats from under test insect tissue. The extraction of Neem pesticides fat must be extracted because the pesticides are lipophilic in properties. For this purpose following procedure were used.

Soxhlation:

For the extraction of pesticides residues from samples of *Callosobruchus analis* Pulse beetle tissue Holden and Marsden (1969) method was used. A known quantity of sample (100) beetles of each strain treated with Neem pesticides separately. The thimble was then placed in the extractor which was fitted to the bolt head flask containing 60 ml. of n-hexane, which was then fitted with condenser connected to the tap water for cooling. The flask was then placed on a heating mantle. The fat extracted solvent was then reduced to about 1 ml evaporation. The process of extraction was carried out for two hours during which all the pesticide residues must have been extracted with solvent. Better recoveries were noticed by this method. Weighing about mg Marcerated with anhydrous sodium sulphate (Na2So4) and was transferred into a thimble made filter paper. For complete recovery of pesticides the column chromatography (sorption) was employed and material was passed three to four time through the columns given below.

Sorption:

This process of sorption was carried out in chromatographic columns of alumina following Holden and Marsden 1969, Silica (Kadoum 1967, 1968)

Alumina and Silica columns:

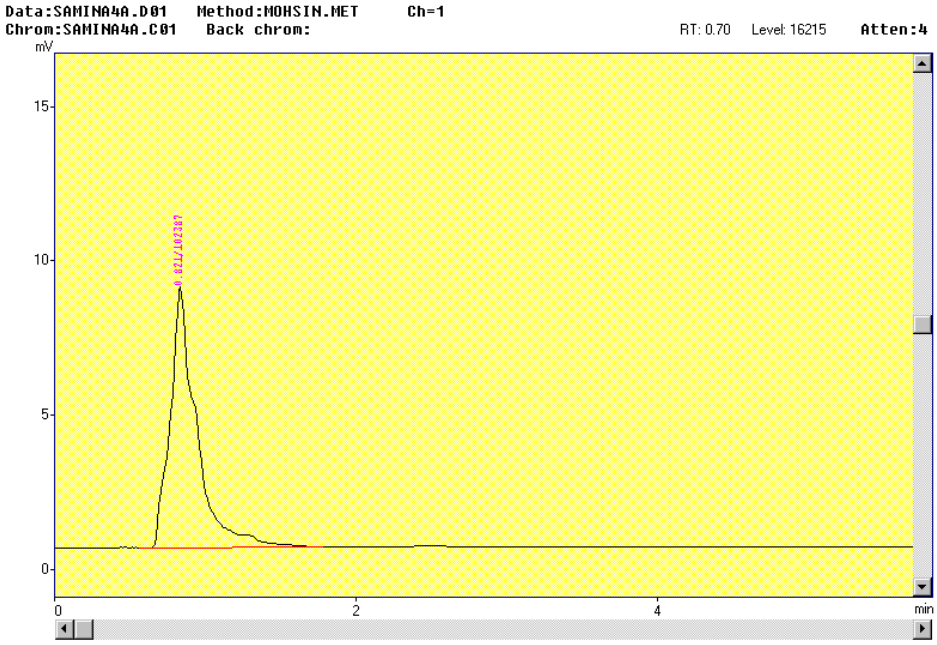
All the fraction were concentrated and make up to / ml separated and identified by HPLC. The same procedure was adopted using the different quantities of standard Neem per ml of n-hexane which are 2µg, 4µg and 6µg in case of Neem It was used to obtain standard peaks for comparison with the samples. Standard chromatograph were prepared by mixing standard. Neem Pesticides for comparison with chromatograms obtained. The alumina column was prepared as and Goternative method for the separation of fat from pesticides which was used by Holden and Marsden (1969). The column was made by glass column having length 40-42 cm with internal diameter 6mm. The concentrated extract was re dissolved in 1 ml of n-hexane (fractional) and transferred on the surface of the alumina column. The column was filled with 2 grams of alumina without calcium of 0.3 micron size already, activated at 800 °C for 4 hours inn furnace and then partly deactivated by shaking with 5% by weight of water.

Now the pesticide adsorbed on the column were eluted with 12 ml of n-hexane and volume of eluted sample was then reduced to 1 ml which was passed through a new column. The new column of the same size was packed with 2 gram of silica gel for column chromatography No, 60, 0.060 millimeter size, activated at 120 °C for 2 hours, cooled and deactivated with 3.5% distilled water. For the removal of trace of moisture layer of activated Na2 So4 was set on top of the silica gel. The elution of pesticides was done by 5 ml of n-hexane and then with 12 ml of 10% diethyl ether in hexane

RESULTS:

SAMPLE INFORMATION:

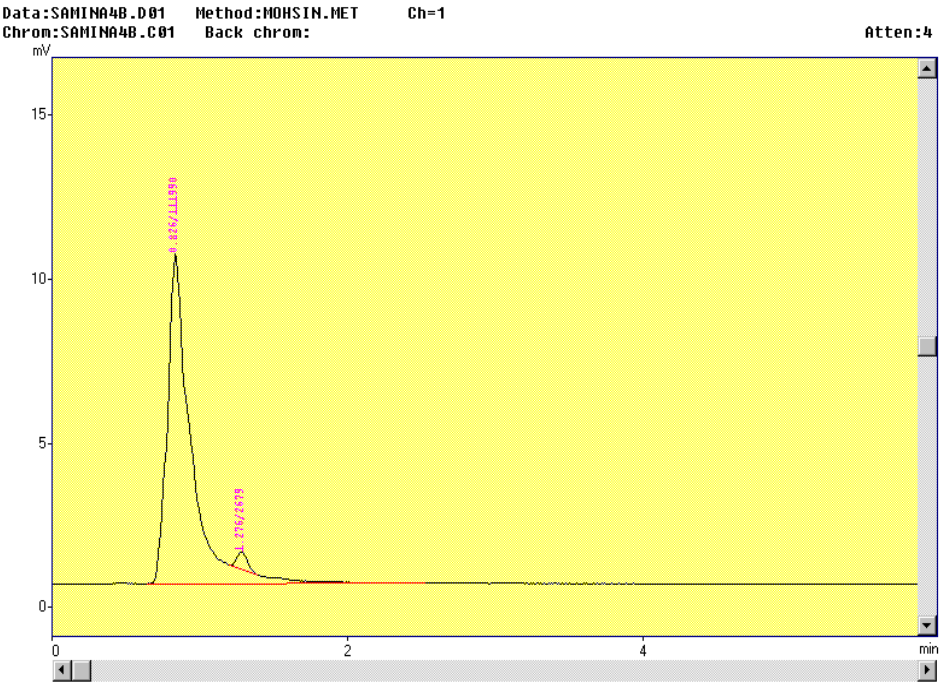
Sample name: Neem Seed Extract (neem component of Biosal) Azadirachtin (1)
Direct Application Method



PEAK RESULTS:

S. No	Peak. No	RT in min	Area in µm
1.	1	0.821	102387
2.	2	Absent	-
3.	3	Absent	-

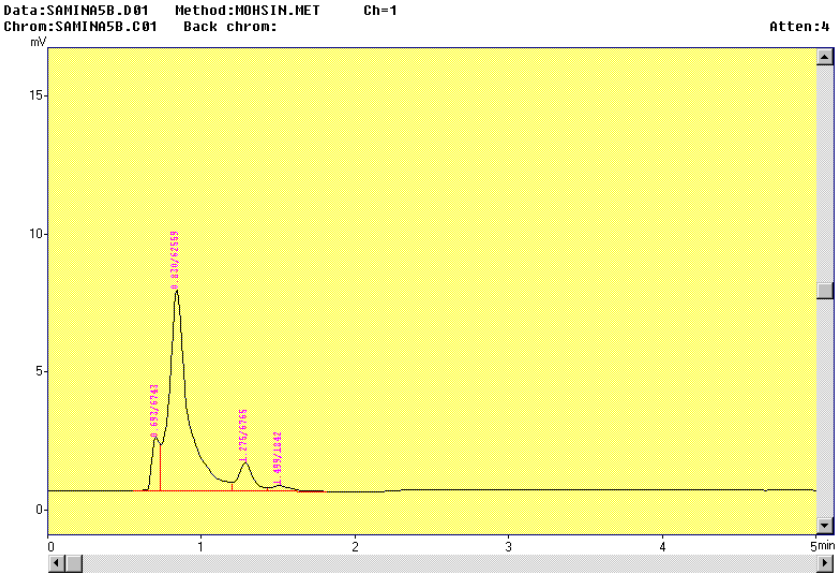
SAMPLE INFORMATION Sample name: Biosal sprayable (2)
Direct application method (sample)



PEAK RESULTS

S.No.	Peak. No	RT in min	Area in µm
1.	1	0.826	111990
2.	2	1.276	2679
3.	3	Absent	-

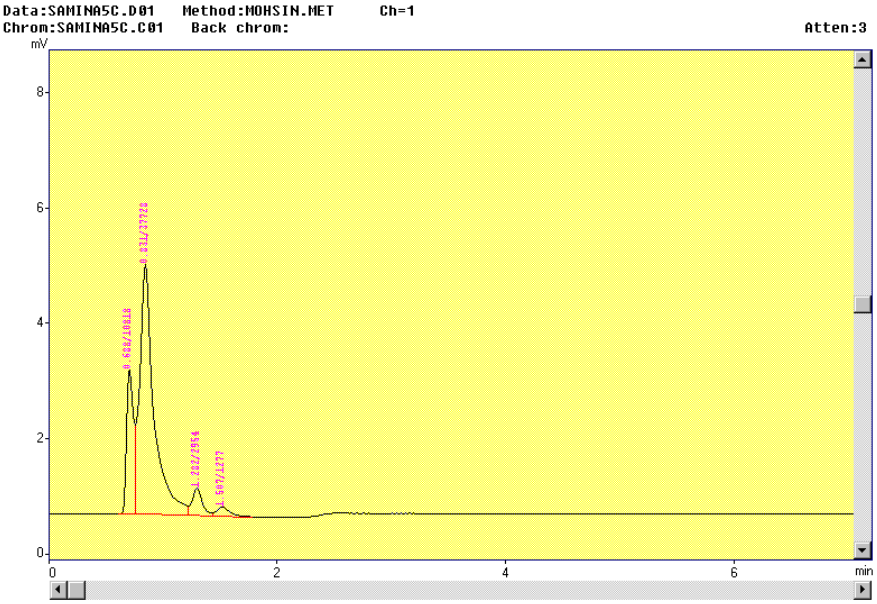
SAMPLE INFORMATION:
Sample name: Biosal sprayable (3)
Glass film method (standard)



PEAK RESULTS:

S.No.	Peak No.	RT in min	Area in µm
1.	1	0.693	6743
2.	2	0.830	62559
3.	3	1.275	6765
4	4	1.499	1842

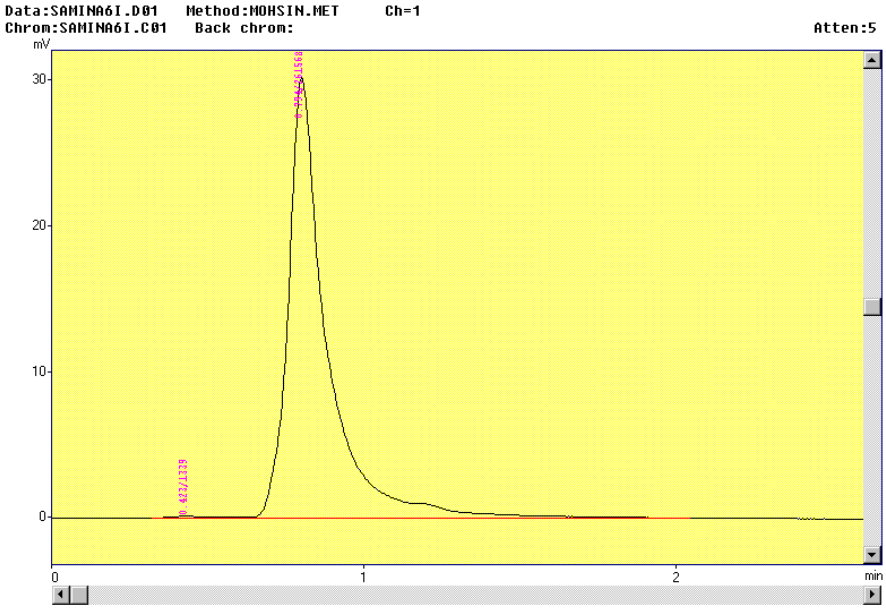
SAMPLE INFORMATON:
Sample name: Biosal sprayable (4)
Glass film method (sample)



PEAK RESULTS:

S.No.	Peak No.	RT in min	Area in µm
1.	1	0.688	10818
2..	2	0.831	37728
3.	3	1.282	2954
4.	4	1.507	1277

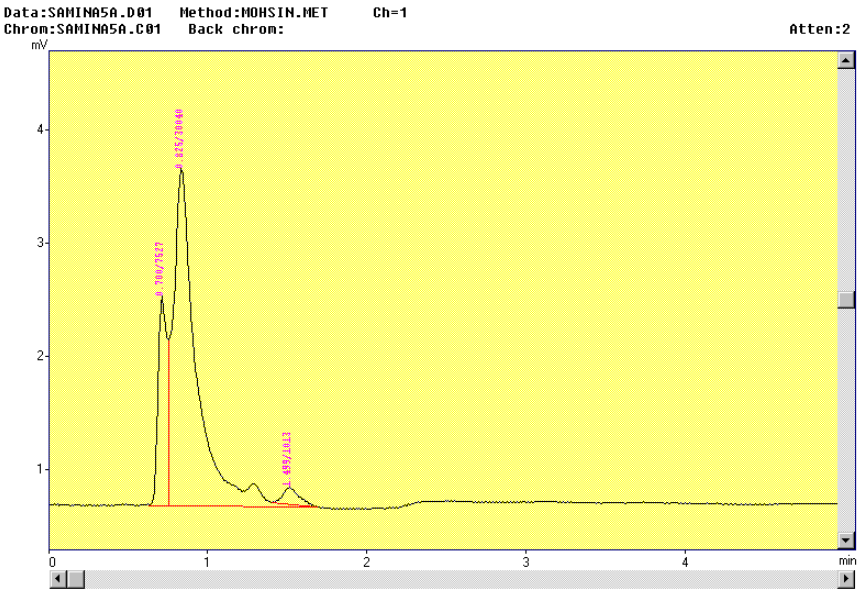
SAMPLE INFORMATION:
Sample name: Azadirachtin (5)
Filter paper impregnation method (standard)



PEAK RESULTS:

S.No.	Peak No.	RT in min	Area in µm
1.	1	0.423	1339
2.	2	0.794	161568
3.	3	Absent	-

SAMPLE INFORMATION:
Sample name: Biosal sprayable (6)
Filter paper impregnation method (sample)



PEAK RESULTS:

S.No	Peak No.	RT in min	Area in µm
1.	1	0.700	7527
2.	2	0.825	30040
3.	3	1.499	1013

Chromatogram No. 1 Azadirachtin (DAM): It shows only one peak i.e Azadirachtin (Standard).
Chromatogram No. 2 Biosal sprayable (DAM): There is one major peak (1) of Azadirachtin and one minor peak which may be twin 80.

Chromatogram No. 3 Biosal sprayable (GFM): The sample run on HPLC shows similar pattern. There is a smaller peak (1) which may be solvent or some other component peak (2) relates to active ingredient i.e Azadirachtin, while peak (3) and (4) may be some biodegradable component.

Chromatogram No. 4 Biosal sprayable (GFM): There is one smaller peak (1) prior to Azadirachtin peak (2) and peak (3) which may be twin 80.

Chromatogram No. 5 Biosal Azadirachtin (FIM): There is only one major peak of azadirachtin and no other peak.

Chromatogram No. 6 Biosal sprayable (FIM): In this case Biosal sprayable was used as azadirachtin peaks have been shown earlier. The chromatogram shows one (1) smaller peak before azadirachtin (2) the major peak which may be of solvent or some other component. In the end one minor peak (3) as shown earlier.

HPLC analysis showed the activity of Biosal against *C.analis* by (DAM) represented by peak area in µm. The seed extract Azadirachtin (standard) shown by one peak 102387, peak (2) and (3) were absent and in Biosal sprayable (sample) shown by two peaks 111990, 2679, peak (3) was absent, while by (GFM) Biosal sprayable (standard) shown by four peaks showed as 6743, 62559, 6765, 1842 µm and in Biosal sprayable (sample) shown by four peaks which was 10818, 37728, 2954, 1277 µm, similarly by (FIM) Azadirachtin (standard) shown one peak 1339, peak 61568 and (3) was absent and Biosal sprayable (sample) shown as 7527, 30040, 1013 µm.

DISCUSSION

Schaaf *et al.* (2000) worked on the reserved-phase HPLC and atmospheric pressure chemical ionization mass spectrometry, a High performance liquid chromatography-MS method was developed to permit the rapid qualitative and quantitative detection of Azadirachtin and related tretranotriterpenoids from tissue culture and seeds of (*Azadirachta indica*). Present work was comparable chromatogram (7), (11) shows by peak i.e Azadirachtin (standard) although the method was slightly different.

Haddad *et al* (1989) were conducted the grain with methanol, hexane and acetone as extracting solvents. Acetone was the best of these solvents because it provided quantitative extraction of the pesticides over a 48-h period, and did not give high levels of ballast material. Clean-up of acetone extracts was accomplished with either Florisil or alumina pre-columns, and up to a tenfold pre concentration was achieved by adsorption of the pesticide on a C_{18} pre-column, or by concentrating the extract through evaporation of the solvent. clean-up and pre concentration were required. Pyrethroids present in the extract at levels in excess of 0.5 µg/ml could be determined by direct injection, but at lower concentrations.

In the present study HPLC analysis showed the activity of Biosal against *C.analis* by (DAM) represented by peak area in µm. The seed extract Azadirachtin (standard) shown by one peak 102387, peak (2) and (3) were absent and in Biosal sprayable (sample) shown by two peaks 111990, 2679, peak (3) was absent, while by (GFM) Biosal sprayable (standard) shown by four peaks showed as 6743, 62559, 6765, 1842 µm and in Biosal sprayable (sample) shown by four peaks which was 10818, 37728, 2954, 1277 µm, similarly by (FIM) Azadirachtin (standard) shown one peak 1339, peak 61568 and (3) was absent and Biosal sprayable (sample) shown as 7527, 30040, 1013 µm. The present study was not comparable may be due to different pesticides.

Rai *et al* (2008) collected grain samples collected from different centers of Kumaon region of State of Uttarakhand. Determined the residues of carbaryl pesticide in surface water, milk, poultry eggs and meat, green fodder and Residue detection of carbaryl was done by High performance liquid chromatography using UV-VIS detector at wavelength 280 nm. The highest mean residual concentration (mg/ml or g) of carbaryl was 0.0527 mg/ml in water followed by 0.0541 in meat, 0.0506 in poultry eggs, 0.0453 in milk, 0.0435 in food grain, and 0.0344 in green fodder. In the present study pesticides observe in stored grain pests. Tamilselvan *et al* (2014) worked on bispyribac sodium 10 % SC 200 g a.i./ha, 500 g a.i./ha and control (water spray) was sprayed using hand operated Maax battery sprayer with a spray volume of 300 litres per hectare at 15 days after transplantation of rice crop (*ADT 45*). At harvest, samples of grain, straw and soil were collected replicate wise from each treatment along with the control. All the residues samples were detected for Bispyribac sodium content by a validated High performance liquid chromatography method at the minimum analysis concentration of 0.01 ppm. These samples were stored in icebox and transfer to the laboratory under cooled condition for detection. In the present study HPLC analysis after 24 hours treatment on Biosal against stored grain pest, *Callosobruchus analis*. More ever three methods of application were also used e.g (a) Direct application method (DAM) on pest in known volume 0.15 ml by Atonizer (b) Glass film method (GFM), where the pesticide in known volume was sprayed on the inner surface of petri plate and 100 insects where release in it (c) Filter paper Impregnation method (FIM) where sample was sprayed in known volume by tower sprayed on filter paper, then 100 insects were released in 90 mm petridish. Peak area in µm and RT in min.

Kowalska (2008) the laboratory experiments was to assess the influence of different forms of azadirachtin (A and B) and the entomopathogen on physiological development and mortality of the insect. Mortality after treatment ranged between 86–93%. There were significant differences in the mean number of surviving stages of the insect between Neem and *Beauveria* treatments.

In the present study toxicity of Biosal essential oil against *Callosobruchus analis* by three methods Direct Application method (DAM), Glass film method (GFM) and Filter paper impregnation method (FIM) after 24 hours of treatment. The LC_{50} value calculated as 1.338504 µl/cm^2 by DAM, 12.174 µl/cm^2 by GFM and 6.945603 µl/cm^2 by FIM, the present study was not comparable may be due to different insects used for mortality.

CONCLUSION

To conclude that the three method, Direct application method (DAM), Glass film method (GFM) and Filter paper impregnation method (FIM) . Biosal (Neem formulaion) (phytopesticide) which seems to be correct on the basis of LC 50 values and HPLC analysis. Possibly, conclude that the pesticides obtained from neem and other plants and trees are beneficial and safer for our environment. Therefore the biosecurity may be provided to the environment by giving preference to bio pesticide.

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