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STABILIZATION OF AZADIRACHTIN IN NEEM OIL USING *PROSOPISJULIFLORA*(LEGUMINOSEAE) AS A BOTANICAL SYNERGIST

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ABSTRACT

This study presents the consequence of P.Julifora, a waste weed plant as a botanical synergist onazadirachtin content inneem oil. During the incubation period of 15 days at 40^{o}c in the laboratory, percentage degradation of azadirachtin and variation in pH of neem oil was observed. After incubation, the azadirachtin content of the neem oil was found to be directly proportional to the concentration of the botanical synergist. The concentration of azadirachtin in neem oil with and withoutbotanical synergist range between 75-31% of the initial concentration. The pH of same sampleswas also observed and it showed a variation from 6.5 to 8.5. After six months, HPLC analysis of the 15th day samples stored at 4^{o}C showed 21.56% to 3.05% decrease in azadirachtin content with increase in the concentration of botanical synergist. Botanical synergist used in this study showed a significant (P<0.002) effect on the stability of the azadirachtin and pH of the neem oil. ATR-FTIR analysis also signifies the stability of Azadirachtin content in neem oil. Thus, various types of stable and eco-friendly formulations of neemoilcan be formed by using a botanical synergist.

Keywords: Neem oil, Azadirachtin, Botanical synergist, Stability, HPLC, ATR-FTIR.

1. Introduction

Neem (*Azadirachtaindica*) belongs to the botanical family of Meliaceae, is a native tree of Mayanmar. An evergreen magical tree grown well in almost all types of soil environment.

Of all neem extracts, the neem seed oil is the best known and the most popular product.Neem oil, a natural soil conditioner, extracted from the seed of the neem tree and have insecticidal and medicinal properties due to which it has been used in pest control. As a botanical pesticide, neem oil is highly valued by organic gardeners around the world because of its simple mode of preparation, locally renewable, user-friendly and environmental safety.

These properties of neem oil were attributed to their secondary metabolites which are triterpenoids and non-terpenoids (Finar 1986; Hellpap and Dryer 1995). Azadirachtin (Figure 1), an active ingredient present in neem oil consist of azadirachtin A at concentration up to 80% and azadirachtin B (3-Tigloylazadirachtol) up to 20%, other compounds such as nimbin (II) and salannin (III) occur at much lower concentrations. (Rembold 1989). The bioactivity of azadirachtin against over 700 pests and disease pathogens has been documented (Yaradua 2007).

Recent advances in azadirachtin research,communicate a new field trial figures by using commercial and semi-commercial provisions of neem oil, against various phytophagous insects and their relatedbeneficial.Investigationsare also made about stored grain pests and insect vectors of various diseases. An increase in the understanding about the chemistry of azadirachtins and their natural and synthetic analogues is leading to important studies on the relationships between their structure and activity and also a breakthrough in the synthesis of its parent molecule (Ley et al., 1993).

Innumerable research works have been done regarding the insecticidal activity and stability of azadirachtin. Stability of azadirachtin was evaluated under various conditions i.e. solvent, pH, temperature etc. (Jarvis et al., 1998; Barreket al., 2004; Madaki 2015). Seasonal variation in the stability of azadirachtinwas also studied (Gupta et al., 2010). Nonetheless,numerous formulations having azadirachtin as an active ingredient isaccessible commercially, but the stability of azadirachtin is a still concern. It is highly unstable under various conditions which is a serious limitation for the development of commercial products. Because of the instability ofazadirachtin under various conditions, its conversion into commercial product has been slow.

A number of products now exist where stability problems have been occurringand possible measures have been done to overcome this.Recent advancement has been done to increase the stability of azadirachtin by using chemical based synergist (Kumar et al., 2003; Gupta et al., 2010; Bandyopadhyayet al., 2014). Although, they enhance the activity and stability of azadirachtin while on the other hand due to chemical based they also have their adverse effect on plants and humans (Bernard and Philogene1993; Falk and Kotin 2006).

To overcome the toxic effect of these chemical synergists, we switch towards the botanicals. This study first time presents an approach to enhance the stability of azadirachtin content in neem oil by using *P.Julifora* as a botanical synergist. In this work, we observe the synergistic effect of botanical synergist on stability of azadirachtin content in neem oil by using HPLC and ATR-FTIR technique.The aim of the present study is to develop various formulations of neem oil i.e. EW, ME, NE, CS and other possible formulations with less degradation of azadirachtin by using botanical synergist as a stabilizer which are less expensive, efficacious and a safe alternate to synthetic, persistent and toxic agro chemical based conventional formulations for pest management.

2. Material and Methods

2.1 Chemicals

Neem oil (Test sample from Gujarat), *P.Julifora*as a botanical synergist , HPLC grade solvent i.e. Acetonitrile, Methanol and HPLC grade water were obtained from Merck, France, Azadirachtin standard was purchased from Sigma-Aldrich, France.

2.2 Instruments

Spinner, Glass vials (Borosil), Rotary evaporator (Buschi), HPLC (Perkin Elmer Series 200 HPLC), ATR-FTIR (Bruker alpha ATR-FTIR).

2.3 Experimental set up

2.3.1 Incubation of neem oil with botanical synergist

Extensive experimental studies were conducted to identify the botanical synergist which can stabilize azadirachtin, an active component in neem oil keeping the final compound as biodegradable. By performing these studies, *P.Julifora*which is abundantly available on waste land throughout India was identified and found that the combination of this synergist with neem oil stabilizes the azadirachtin content in the neem oil thus also enhance its insecticidal activity. For optimization, the most effective ratio number of experiments were conducted. To study the synergistic effect of botanical synergist on the stability of azadirachtin present in neem oil, five different concentrations i.e. 0g, 1g, 2g, 6g and 10g were taken in a 250ml Erlenmeyer flask and mixed with 100 ml of pure neem oil. Each flask was incubated at 40^{o}C at 150rpm in an incubator shaker for 3hr. After the incubation period, neem oil from each flask were filtered through wattman filter paper in a fresh 250ml Erlenmeyer flask.

2.3.2 Enrichment of neem oil with Azadirachtin

100g technical grade azadirachtin were added in each 250 ml Erlenmeyer flask having filtered neem oil and botanical extract. 3ml samples from each flask were pipette out in a glass vial after 0^{th} , 1^{st} , 3^{rd} , 5^{th}, 10^{th} and 15^{th} day of incubation.

2.3.3 pH determination of neem oil

pH of each sample was also determined after each interval of incubation period using a Mettler Toledo pH meter (digital). This was carried out in triplicates at a temperature range of $25^{o}C$ – $30^{o}C$.

2.3.4 Extraction of Azadirachtin from neem oil

Each sample was processed with HPLC grade acetonitrile to observe the azadirachtin content in neem oil remained after particular incubation period. 6ml of acetonitrile were mixed with 3 ml neem oil sample and vortex for 3 min. After vortex, kept them undisturbed for 30 min for the separation of organic and aqueous layer. The upper organic layer having azadirachtin was pippet out in fresh acid washed 100ml round bottom flask. This process was repeated twice more with each and every sample. Total 18ml of acetonitrile were pippet out in a round bottom flask. Each flask was dried in the rotatory evaporator (BUSCHI) to evaporate acetonitrile. Azadirachtin content remained in a round bottom flask were extracted out with 3ml of HPLC grade acetonitrile.

2.4 Evaluation of stability of Azadirachtin

The percentage decrease in the peak area of azadirachtin content in neem oil was determined in triplicate for 15 days in heat storage at $40^{o}C$. The samples were drawn after 0, 1, 3, 5, 10 and 15 days of incubation.

2.5 Quantitative analysis of Azadirachtin content

Azadirachtin extracted with 3ml of HPLC acetonitrile were further used for quantitative analysis of percentage degradation in azadirachtin during the experiment. Using this extract further dilution of 50ppm was prepared using HPLC grade acetonitrile and 10ul of which was injected for HPLC analysis using 25ul syringe.

The operating condition and column specification of HPLC (Perkin Elmer Series 200 HPLC) during the analysis of azadirachtin were: flow rate1.2ml/min, duration of the cycle is 60 min, mobile phase: Acetonitrile: Water (35:65), detector wavelength 214nm, column C-18 and injection volume is 10ul.

2.6 Characterization of Azadirachtin

50ppm of Technical grade azadirachtin (Sigma) was injected in HPLC. Depending upon their retention time (R_t), two peaks were obtained at a particular time for Azadirachtin A (R_t-14.82 min) and Azadirachtin B (R_t-15.88min) respectively (Figure 2). Thus, by comparing the retention time of a particular peak to that of the standard both the metabolites of azadirachtin were identified.

2.7 ATR-FTIR analysis of Azadirachtin in neem oil

Infrared (IR) spectra of each sample were recorded on a Bruker alpha ATR-FTIR spectrophotometer using the attenuated total reflectance (ATR) technique, and values were expressed as gmax cm^{-1}.Pod extract of *P.Julifora* were also processed with 70% of ethanol extraction followed by column purification and IR analysis were done.

2.8 Statistical analysis

Two-way ANOVA (Sigma plot v13.0) was used to determine if there was a significant difference in the percentage degradation of azadirachtin with and without the addition of synergist in neem oil over a 15 days of incubation period.

3. Results

3.1 Effect of botanical synergist on degradation of Azadirachtin

In this work we studied the rate of degradation of azadirachtin with *P.Julifora* as a botanical synergist in neem oil within the regular interval of time. After each interval of time of incubation, percentage degradation of azadirachtin was observed and quantified by HPLC analysis.During the 15 days of incubation period, a significant variation in the percentage degradation of azadirachtin in neem oil was observed (Table 1).

Table 1. Two Way Analysis Of Variance Showing The Significant Difference In The Percentage Degradation Of Azadirachtin In Neem Oil During The 15 Days Of Incubation Period With Different Concentration Of Botanical Synergist Using Days And Amount Of Synergist (G/100ml Neem Oil) As Variables.

Source of Variation	DF	SS	mean square	F value	P value	Std Err of LS Mean
Days	4	0.315	0.0788	12.975	<0.001	0.039
Synergist	3	0.169	0.0562	9.257	<0.002	0.0349

Neem oil with 0g of botanical synergist extract showed significantly (P<0.002) higher

percentage of degradation in azadirachtin content followed by 2g, 6g and 10g (Figure 3). Initially, the azadirachtin content in all the sample flask was same while after 5th day of incubation, flask with 0g of botanical synergist extract showed 32.21% of degradation followed by 20.3%, 19.1% and 19.3% of the neem oil sample with 2g, 6g and 10g of botanical synergist extract respectively. Finally, after 15th day of incubation, a significant ($P<0.001$) variation was observed in the percentage degradation of azadirachtin in neem oil. Flask with 0g of botanical synergist extract showed a 69.65% decrease in azadirachtin content (Figure4). Subsequently, neem oil incubated with 2g and 6g of the botanical synergist extract showed 38.8% and 28.49% decrease in azadirachtin content respectively. Finally, neem oil with 10g of the botanical synergist extract showed only 25.5% of the decrease in azadirachtin content (Figure5).

After 3 months, percentage degradation of these samples were again, quantified by HPLC analysis. Neem oil with 0g of botanical extract showed a 75% decrease in the azadirachtin content while samples with 2g, 6g and 10g of botanical extract showed 50.01%, 30.50% and 28.50% decrease in azadirachtin content in neem oil.

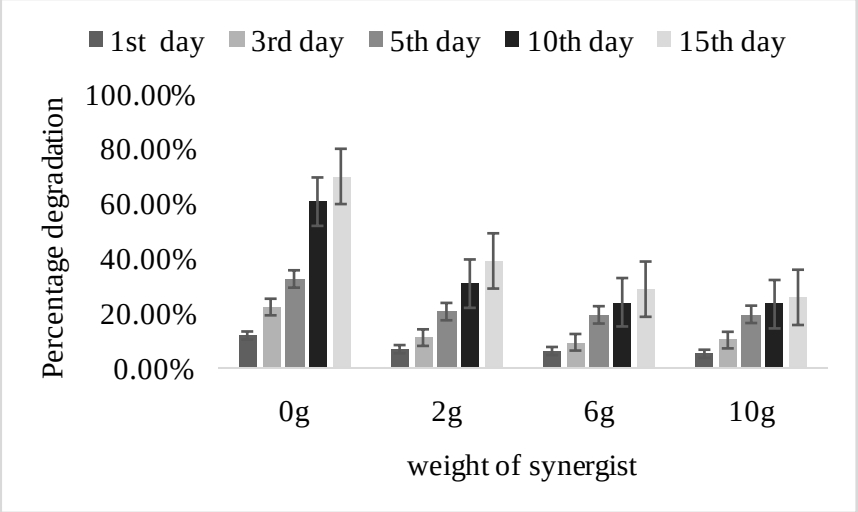

Figure 3. Percentage Degradation In AzadirachtinContent Present In Neem Oil Incubated With Different Concentrations Of Botanical Synergist.

3.2 Rate of Degradation

Half-life ($t_{½}$) of azadirachtin in neem oil with and without different concentrations of botanical synergist was observed and it ranged from 9.5 to 24.2 days (Figure6). $t_{½}$ of azadirachtin contentin neem oil with 0g of botanical synergist was observed to be 9.5 days while with 2g,6g and 10g of botanical synergist was 17.5, 22.6 and 24.2 days respectively.

3.3 ATR-FTIR analysis of neem oil

The absorbance band of neem oil (Figure7) due to azadirachtin were located at 1375 cm^{-1} (C-H branching), 1636 cm^{-1} (carbonyl stretching), 1734 cm^{-1} (ester) and 2855 cm^{-1} (OH stretching). There were no change in the absorbance band of azadirachtin due to the presence of a botanical synergist extract in neem oil (Figure8).In case of *P.Julifora*absorbance band at 3289 cm^{-1}, 2926 cm^{-1} and 2927 cm^{-1}were observedpossibly due to presence of OH stretch band of phenols or alcohols (Figure 9)

3.4 Effect of botanical extract on pH of neem oil

Effect of botanical synergist on the pH of neem oil is graphically represented inFigure10.There were not any significant variation was observed for the 15 days of incubation period, except in the neem oil having 0g of botanical synergist extract.pH of neem oil without any botanical synergist extract varies from 6.5- 8.5 while neem oil with 2g, 6g and 10g of the botanical synergist extract showed pH from 6.5-7.0.

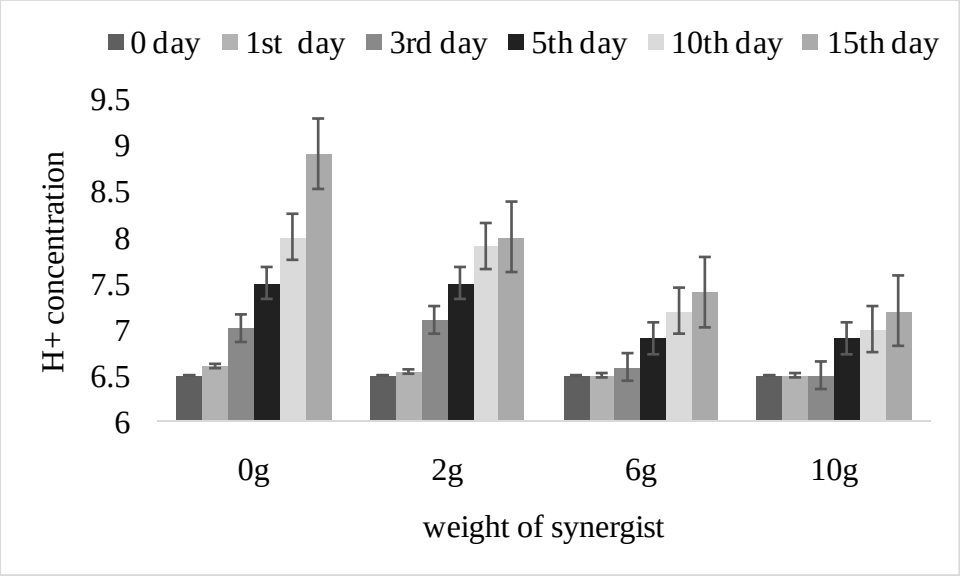

Figure10. Ph Of Neem Oil Incubated With Different Concentration Of Botanical Synergist At The Different Interval Of Time During The 15 Days Of Incubation Period.

4. Discussion

4.1 Effect of botanical synergist on degradation of Azadirachtin

Azadirachtin is an important constituent of neem oil responsible for its insecticidal activity so far. Various research works have been done regarding the insecticidal activity and stability of azadirachtin. In the present work, we studied the synergistic effect of *P.Julifora* as a botanical

synergist on the rate of degradation of azadirachtin in neem oil within the regular interval of time. After each interval of time of incubation, percentage degradation of azadirachtin was observed and quantified by HPLC analysis. A significant variation in the percentage degradation of azadirachtin in neem oil was observed. Neem oil with 0g of botanical synergist extract showed significantly ($P<0.002$) higher percentage of degradation in azadirachtin content followed by 2g, 6g and 10g (Figure 3). After 3 months, percentage degradation of these samples were again, quantified by HPLC analysis and observed that presence of botanical synergist decrease the rate of degradation of azadirachtin. This also signifies that, botanical synergist used in this

study maintain the long term stability of azadirachtin in neem oil.This result suggested that the increase in the amount of botanical synergist showed a significant ($P<0.001$) decrease in the percentage degradation of azadirachtin in neem oil. This decrease might be due to the presence of glycosidicand phenolicscompound present in botanical extract

(Table 2)(Barros et al.,1981;Woźniaket al., 2014).

Table 2. BromatologicAnalysis Of *P. Juliflora* Pods Obtained By Barros Et Al., (1981) (FAO).

Components	**Percentage (%)**
Raw Protein	12.93
Digestible Protein	9.77
Calcium	0.42
Phosphorus	0.18
Soluble Carbohydrates	54.16
Aminogram	
Alamin	0.17
Arginin	0.23
Aspartic Acid	0.25
Cystine	0.05
Phenilalamin	0.09
Glicine	0.21
Glutanic Acid	0.44
Isoleucine	0.13

Kumar et al., (1999) studied the effect of different stabilizer along with carrier on the rate of degradation of azadirachtin in neem oil. They observed that using anthraquinone and epichlorhydrin as a stabilizer and clay based powder as a carrier improves the shelf-life of azadirachtin-A. Among these, two compounds, anthraquinone is a naturally occurring phenolic compound and have a mutagenic effect on DNA (Vasil'eva 1989). While epichlorhydrin is a highly reactive compound used in the manufacture of plastics, elastomers etc. and also have carcinogenic effect on human health (http://apps.sepa.org.uk/spripa/Pages/SubstanceInformation.aspx?pid=48).

Shrivastavaet al., (2011) worked on the enhancement of azadirachtin by using various chemical synergist i.e. tween-20, triton X-100, para-amino benzoic acid (PABA), anthraquinone, 8-hydroxy quinoline, ter-butyl hydroquinone. Although, they enhance the stability and efficacy of azadirachtin however all the synergist used in this study have toxic effect on plant and human health. Similarly, Damralaet al., (2007) prepare a storage stable formulation of azadirachtin A by using various solvent. In this work, they studied the degradation of azadriachtin A in various solvents for 25 days at 29^{o}C ±3 indicated that 50% of degradation of azadirachtin A in methanol and acetone, 75-80% in methylene chloride, carbon tetrachloride and chloroform and about 85% in ethanol and water. All these solvents are well known for their toxic effect. Whereas, in the present study, naturally occurring botanical synergist was used to enhance the stability of azadirachtin in neem oil which is quite safe for plants as well as for human health.

4.2 Rate of Degradation

Half-life ($t_{½}$) of azadirachtin in neem oil with and without different concentrations of botanical synergist was observed and it ranged from 9.5 to 24.2 days (Figure 6). The lowest $t_{½}$ value was obtained with 0g of botanical synergist and increases with the increase in concentration of botanical synergist. An increase in the half-life of azadirachtin again signifies its stability due to the presence of botanical synergist.

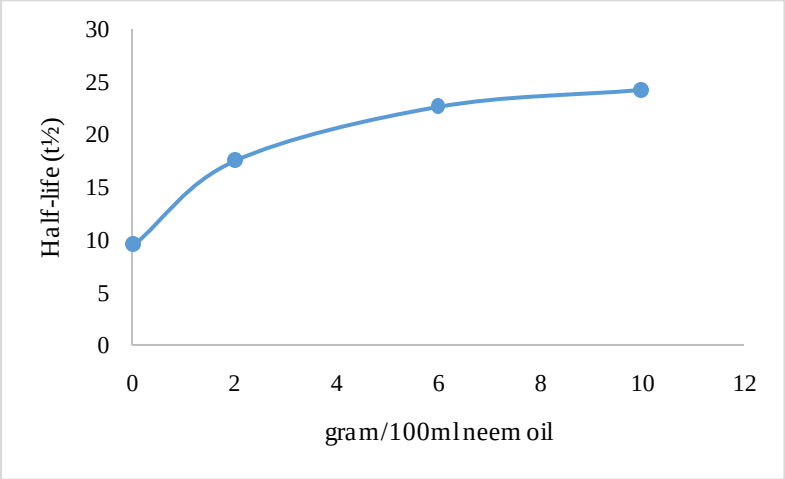

Figure 6. Half – Life (T½) Of Azadirachtin In Neem Oil Incubated With Different Concentrations Of Botanical Synergist.

Kumar et al.,, in 1999, used chemical synergist and observed $t_{½}$ of 26.18days for azadirachtin content in neem oil while in the present study by using botanical synergist we observed $t_{½}$ of 24.2 days. There is no such significant difference in the half-life of azadirachtin content. This also indicates that botanical synergist effectively replace the use of chemical synergist without any significant loss of active ingredient in neem oil.

4.3 ATR-FTIR analysis of neem oil

There were no change observed in the absorbance band of azadirachtin due to the presence of a botanical synergist extract in neem oil (Figure 8). This signifies that the botanical synergist used in this study stabilize the active ingredient of neem oil without changing their chemical properties (Deota and Upadhyay 2005). The spectral analysis of phytochemicals present in the pod extract of *P.Julifora* was carried out by comparing with previous publish literature (Kale et al., 2013).The absorbance band at 3289 cm^{-1},2926 $cm^{-1}$and 2927 cm^{-1}possibly due to presence of OH stretch band of phenols or alcohols which might be responsible to stabilize the azadirachtin content in neem oil. (Figure 9) (Bhatia et al., 2013).

4.4 Effect of botanical extract on pH of neem oil

pH of botanical synergist used in this study was observed to be pH 4.3 might be due to this it act as acidic buffer and maintains the pH of neem oil. In an earlier study, Jarvis et al., (1998) observed that pH 6-7 is to be most favourable for the formulation of neem oil. In the present study, botanical synergist sustains the pH of neem oil, which also signifies the stability of azadirachtin in neem oil.

The interaction between botanical synergist and azadirachtin is well documented. Our earlier work on the stability of azadirachtin in NKP using botanical synergist were also shown similar results (1627/DEL/2009). Various concentrations of botanical synergist and NKP were used to perform different experimental tests and out of these, 1:1 ratio of NKP and botanical synergist gave 100% mortality in the performed experiments. The result showed that, botanical synergist enhances the insecticidal and larvicidal effect of NKP against stored grain pest. The environmental safe mosquito coil was also developed using botanical synergist which is quite effective as compared to commercially available mosquito coil (365/DEL/2010). These experiments were done to stabilize azadirachtin present in the form of neem kernel powder.

5. Conclusion

Thus, the present study indicates that botanical synergist used in this study, significantly enhance the stability of azadirachtin in neem oil for a longer period of time. It also maintains the pH of neem oil from 6.5-7.5 which signifies that neem oil remain effective for a longer period of time with its insecticidal properties. Alcoholicand phenolicscompounds present in the botanical synergist extract might be responsible to enhance the stability of azadirachtin in neem oil.Thus, the present study indicates that by using a botanical synergist which is less expensive, efficacious and free from any toxic compound we will enhance the stability and efficacy of azadirachtinby 75% in neem oil formulation.This new approach help to replace the toxic chemical synergist with a non-toxic naturally occurring botanical plant as a synergist and also develops stable and eco-friendly formulations of neem oil i.e ME, NE,CS etc. for pest management.

Acknowledgement

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Conflict of Interest

There is not any financial conflict of interest.

References

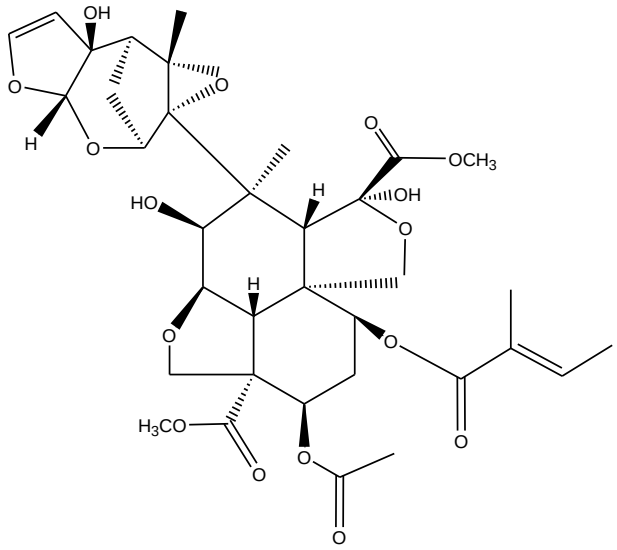
- Bandyopadhyay, W.S., Gotyal, B.S., Satpathy, S., Selvaraj, K., Tripathi, A.N. and Ali, N.(2014),“Synergistic Effect of Azadirachtin and Bacillus thuringiensis against Bihar Hairy Caterpillar, Spilarctia oblique”,Biopesticide International,Vol. 10, pp. 71-76.
- Barrek, S., Paisse, O., Florence, M. and Loustalot, G. (2004), “Analysis of neem oils by LC–

MS and degradation kinetics of azadirachtin-A in a controlled environment Characterization of degradation products by HPLC–MS–MS", Analytical and Bioanalytical Chemistry, Vol. 378, pp. 753–763.

- Bernard, C.B. and Philogène, B.J.(1993), "Insecticide synergists: role, importance, and perspectives", Journal of Toxicology and Environmental Health, Vol. 38, pp. 199-223.
- Bhatia, H., Gupta, P.K. and Soni, P.L.(2013), "Extraction purification and characterization of a galactomannan from Prosopisjuliflora (SW.) DC seed polysaccharides", International journal of Science Environment and Technology,Vol. 2,pp. 708-724.
- Barros, N.A.M.T., Nobre, F.V.,Azevedo, C.F.de., Barbosa, C.A.N. and Brandao, J. do N.(1981), "Algarobeira, importanteforrageira para o Nordeste," Natal, emparn, PP.34.
- Damrala, S.R., Sridhar, S., Gopinathan, C.M. and Raman, K. (2007),"A storage stable pesticide formulation containing azadirachtin". Application no. 507/MAS/2000.
- Deota, P.T. and Upadhyay, P.R. (2005),"Biological studies of azadirachtin and its derivatives against polyphagous pest, Spodopteralitura", Natural Product Research,Vol. 19, pp. 529-539.
- Falk, H.L. and Kotin, P.(1969)'"Pesticide Synergists and their metabolites: Potential Hazards", Annals of the New York Academy of Sciences,Vol. 160, pp. 299–313.
- Finar, I.L. (1986), Stereochemistry and the chemistry of natural products. Organic chemistry, Longman Publishers, England.
- Gupta, V.K., Ahlawat, S.P., Kumar, R.V., Ajit. and Dutta, A.(2010),"Effect of season and year on Azadirachtin A and oil content in neem (Azadirachtaindica A. Juss) seeda and relationship of Azadirachtin A and oil content with rainfall, temperature and humidity", Current science,Vol. 99, pp. 953-956.
- Hellpap, S. and Dreyer, M.(1995),The smallholders' neem products. In: Schmutterer, H. (Eds.,), Neem tree: Source of unique natural products for Integrated Pest Management, Medicine, Industry and other purposes; (ed.) Weinhein, Germany, pp. 367- 375.
- Jarvis, A.P., Johnson, S. and Morgan, E.D.(1998), "Stability of the natural insecticide azadirachtin in aqueous and organic solvents", Journal of Pesticide Science,Vol. 53, pp. 217-222.
- Kale, M.S. and Laddha, K.S.(2013),"Isolation, characterization and quantification of isoflavone in *Momordicadioica*roxb. Ex wild (*cucurbitaceae*) fruits",International Journal of Applied Research in Natural Products, Vol.5, pp. 28-34.
- Kumar, A. R. V., Jayadevi, H. C., Ashoka, H. J. and Chandrashekara, K. (2003),"Azadirachtin use efficiency in commercial neem formulations" Current Science,Vol. 84, pp. 1459-1464.
- Kumar, J. and Parmar, B.S. (1999), "Stablization of Azadirachtin A in neem formulations: effect of some solid carriers, neem oil and stabilizer", Journal of Agriculture Food and

Chemistry,Vol. 47, pp. 1735- 1739.

- Ley, S.V., Denholm, A. A. and Wood, A.(1993),"The chemistry of azadirachtin", Natural Product Report, Vol.10, pp. 109-157.
- Madaki, F.M. (2015), "Studies on the Effect of Temperature, Light and Storage on the Stability of Neem (AzadirachtaIndica A. Juss) Seeds Oil Extract", IOSR Journal of Pharmacy and Biological Sciences,Vol. 10, pp. 46-50.
- Patanjali, P.K.(2011),"Synergistic Insecticidal and Larvicidal Botanical Compositions", Application No. 1627/DEL/2009.
- Patanjali, P.K. (2012), "A novel synergistic mosquito repellent composition for preparation of mosquito coils", Application No. 365/DEL/2010, International Classification No. A01N31/14.
- Rembold, H.(1989),"Azadirachtins: their structure and mode of action", In: Arnason, J. T., Philogene, B. J. R. and Morand, P. (Eds.,), Insecticides of Plant Origin, American Chemical Society, Washington, DC, pp. 150-163.
- Scootish environment protection agency, Epichlorhydrin. http://apps.sepa.org.uk/spripa/Pages/SubstanceInformation.aspx?pid=48 (accessed 1.06.2016).
- Srivastava, M. and Srivastava, A.K. (2011),"Stability enhancement of Azadirachtin (a biopesticide) produced in vitro against atmospheric degradation", Bio Tech,doi: 10.5176/978-981-08-8119-1.
- Vasil'eva, L.A., Pylev, L.N., Sikora, M., Guretska, D. and Gurska, E.(1989),"Mutagenicity and harmful effects of anthraquinone dyes on DNA",GigienaTruda I Professional'NyeZabolevaniia,Vol. 5, pp. 28-30.
- Yaradua, A.A.(2007), "Potentials of biopesticides from neem tree (Azadiractaindica A. Juss) in sustainable pest and disease management in Nigeria", In: Medicinal plants in Agriculture. Proc. Akure-HumboltKellog 3rd SAAT Annual Conference 2007, pp. 237254.
- Woźniak, L., Marszałek, K. and Skąpska, S. (2014),"Influence of Steviol Glycosides on the Stability of Vitamin C and Anthocyanins",Journal of Agriculture Food Chemistry,Vol. 62, pp. 11264–11269.



Azadiarachtin A

Figure 1. Structure OfAzadirachtin A

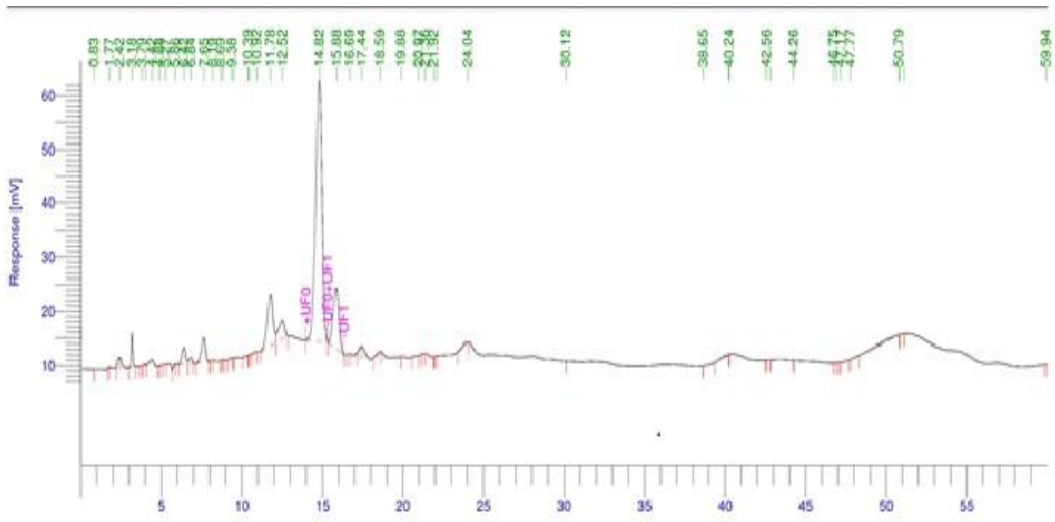


Figure 2.HPLC Chromatogram Showing The RtValue Of Technical Azadirachtin (100ppm).

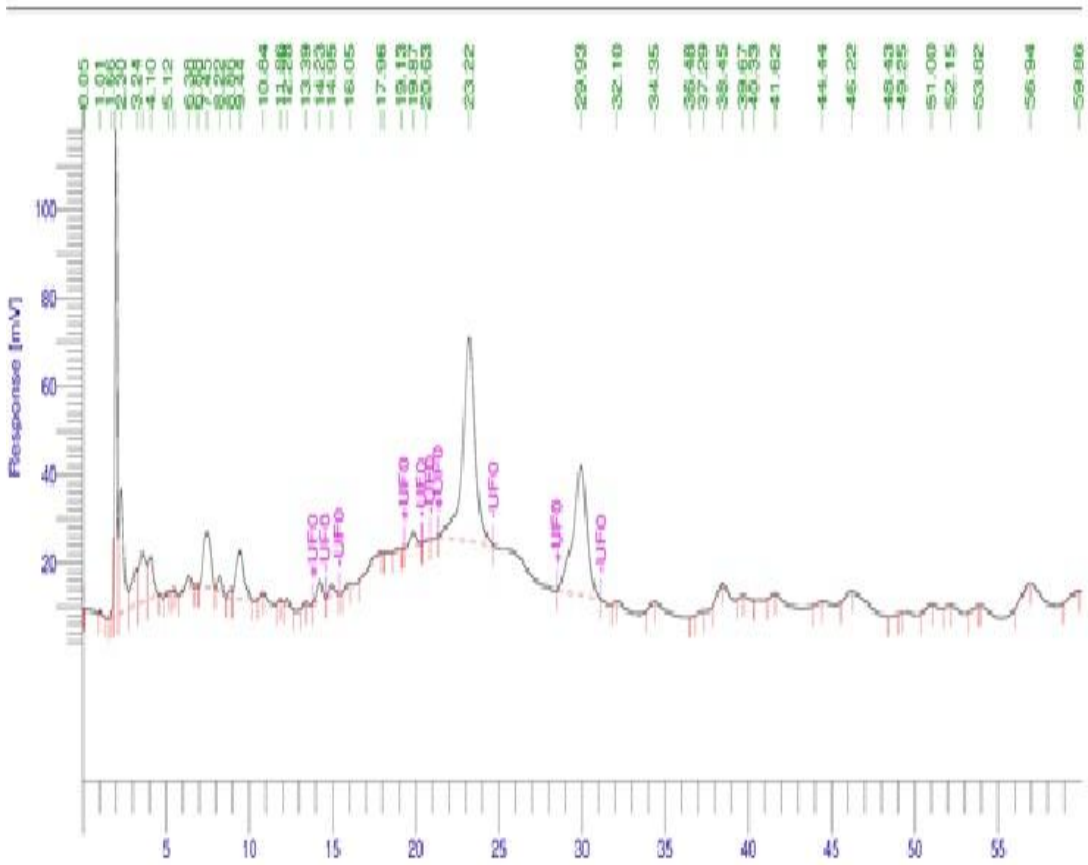


Figure 4.HPLC Chromatogram Showing RtValue Of Azadirachtin Present In Neem Oil Incubated With 0g Of Botanical Synergist Extract After 15th Day Of Incubation Period.

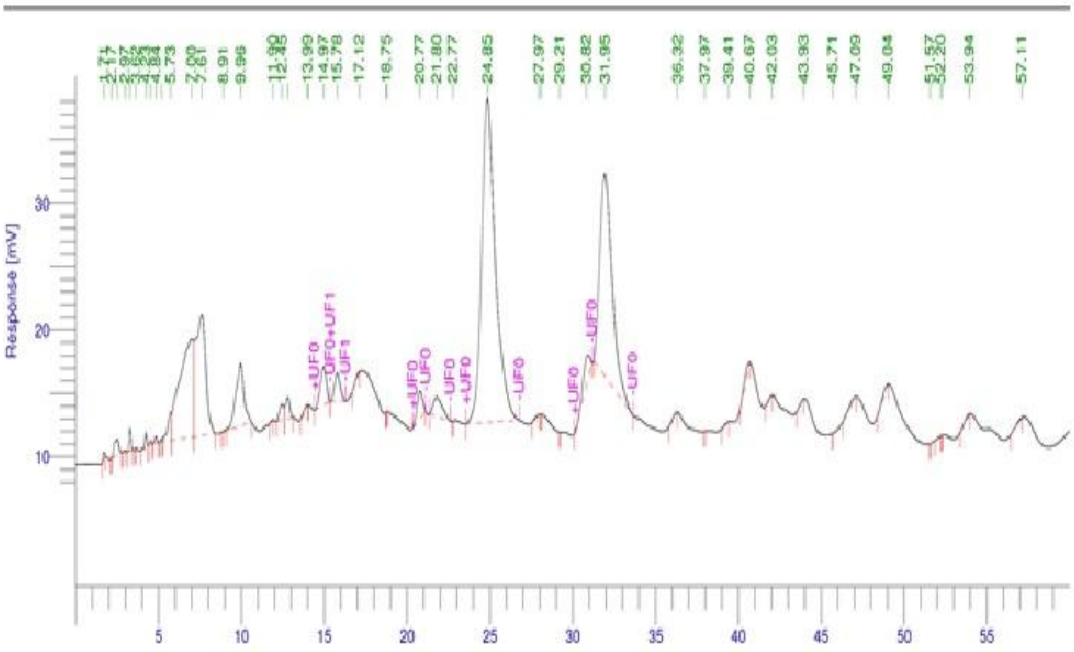


Figure5. HPLC Chromatogram Showing RtValue Of Azadirachtin Present In Neem Oil Incubated With 10g Of Botanical Synergist Extract After 15th Day Of Incubation Period.

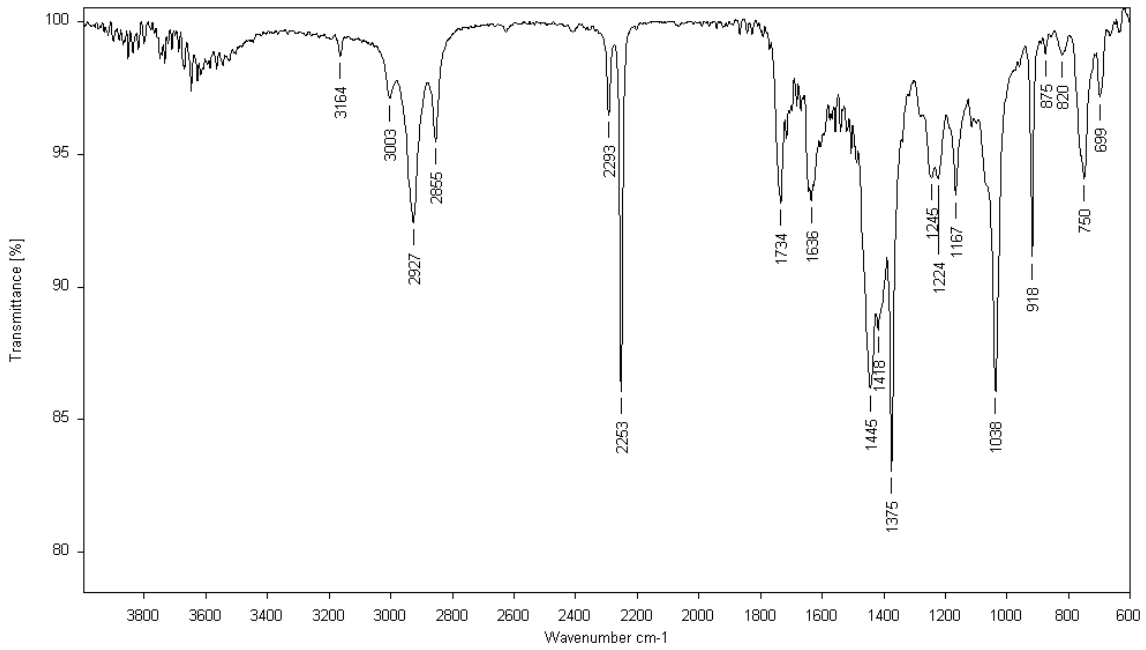


Figure 7.FTIR Chromatogram OfNeem Oil Showing The Characteristic Band Of Azadirachtin.

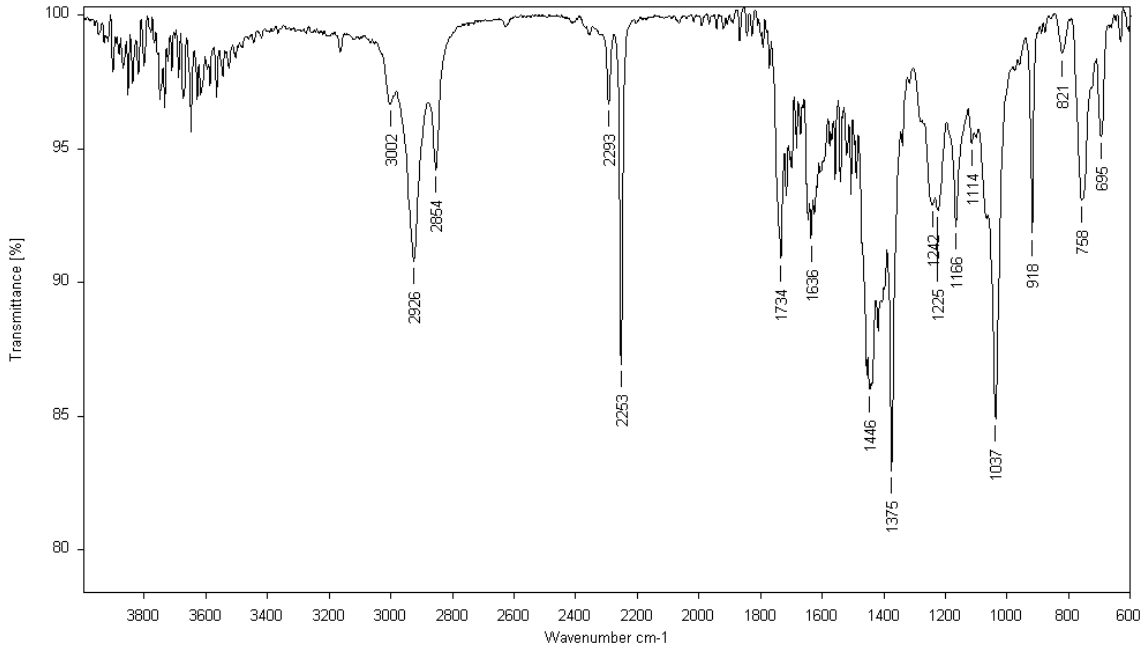


Figure 8. FTIR Chromatogram OfNeem Oil Incubated With Botanical Synergist Showing No Change In The Band Of Azadirachtin.

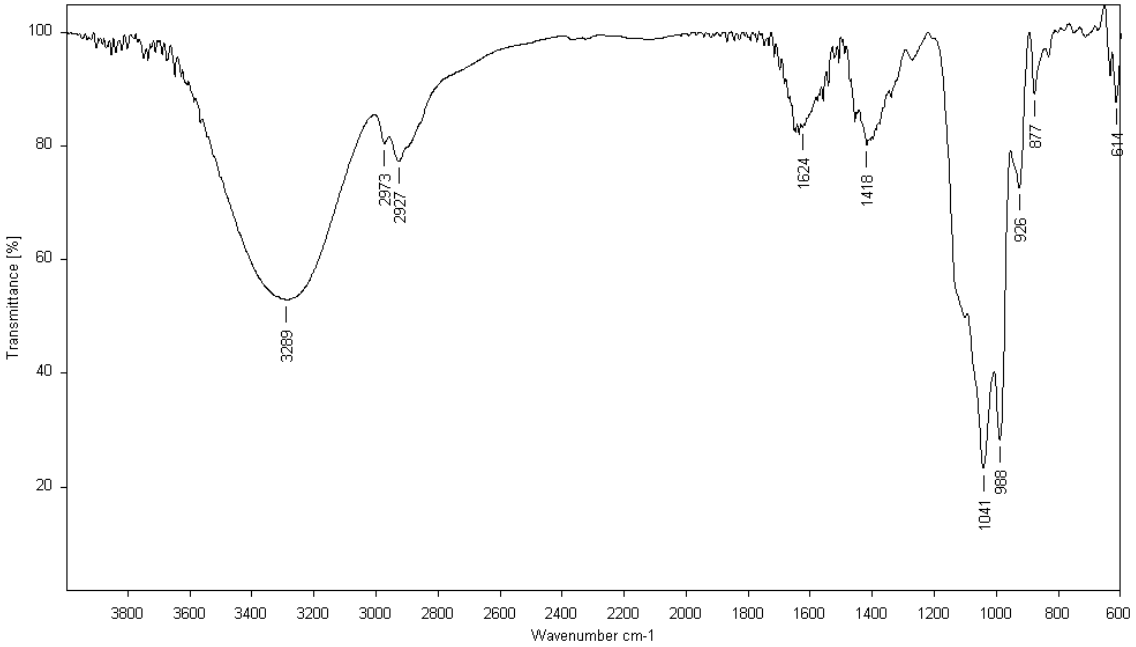


Figure 9. FTIR Chromatogram Of Pod Extract Of *P.Juliflora*Showing The Presence Of Phenolics And Alcoholic Compounds.