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INSECTICIDAL EFFECT OF NEEM (*Azadirachta indica a.juss)* LEAF AQUEOUS EXTRACT ON FLEA BEETLE (*Podagrica spp*) ON ROSELLE (*Hibiscus sabdariffa* L) IN KEBBI STATE-NIGERIA.

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ABSTRACT

The study evaluates the efficacy of the neem leaf aqueous extract NLAE at different graded levels on flea beetle (*Podagrica spp)* infecting roselle (*Hibiscus sabdariffa* L.) varieties in rainy season in 2020 at Aliero and Jega, Kebbi State, Nigeria. The treatment consisted of three varieties of Roselle Green (V1), Red (V2), and deep red (V3) and three graded level of NLAE (C1-1000mg/mL, C2-500mg/mL, C3-333mg/mL) and control (C0-0mg/mL). The experiment was laid out in randomized complete block design (RCBD) in a split plot arrangement and replicated three times. Different graded extracts were sprayed as Roselle flowering reaches 50%. The spraying procedure was repeated at 0 day before spray (DBS), 1, 2, 3, 5 and 7 days after spraying (DAS) with (NLAE). Data were collected on Flea count, number of fresh and dried calyx, fresh and dried leaf yields per hectare (kg/ha). The results revealed that *Podagrica spp* were identified as a major pest complex affecting Roselle production. Furthermore, results also revealed the efficiency of the Neem leaf aqueous at different grade levels on pest affecting Roselle, while untreated control recorded the highest number of *Podagrica spp* with significant difference among the treatment that eventually have great effect of the yield of Roselle.

Keywords: Roselle, *Podagrica, Neem,* Insecticide, insect population

INTRODUCTION

Roselle (*Hibiscus sabdariffa L.*) is one of the most important traditional leafy vegetables (TLVs) in the tropics and sub-tropics, (Ansari, 2013). Especially in India, East Indies, Nigeria and South America (Abiodun et al., 2018). It is believed to have originated from Africa. More than half of these species originate in the warmer parts of central and eastern Africa, while others from tropical America, Asia and Australia (Schippers, 2000). Roselle is a rich source of anti-oxidants, riboflavin, ascorbic acid, niacin, and carotene that are nutritionally important as well as amino acids and mineral salts (Anjah, 2012). The annual production of the crop worldwide is said to be very low; due to insect infestation. Despite these medicinal and food values, Roselle cultivation is limited in Nigeria and farmers plant the crop without due consideration to appropriate planting date and insect infestation (Futuless *et al*., 2010). Insect pests play an important role in limiting crop yield especially in the tropics where the climate favours their production. Insect pests' infestation is one of the major factors militating against cultivation of Roselle., (Fasunwon and Banjo, 2010). Different phonologies of Roselle have been reportedly attacked by insects. Among the insect pests, Major insect pest of Roselle in Nigeria includes *Podagrica uniforma* J., and *P. sjostedti* J., (Fasunwon and Banjo, 2010). Podagrica species cause economic damage, attacking the lamina of the young foliage and matured leaves which result in reduction of the photosynthetic ability of the crop (Fasunwon & Banjo 2010). The commonest pest control method is usually by the application of synthetic insecticides that are unfortunately hazardous to the ecosystem. One of the major synthetic insecticides is Lambda-cyhalothrin. This is a member of a class of insecticides known as pyrethroids (Robert, 2008). Synthetic Pesticides are highly effective, rapid in action, convenient to apply, usually economical and most powerful tools in pest management. However, indiscriminate, inadequate and improper use of pesticides has led to severe problems such as development of pest resistance, resurgence of target species, outbreak of secondary pests, destruction of beneficial insects, as well as health hazards and environmental pollution. It is therefore, a high time to re asses and evaluate the suitable products to be used in plant protection strategy. In an inte- grated control programme, it was necessary to utilize some insecticides with minimal toxicity to natural enemies of pests. Such practice might help to alleviate the problems of pest resurgence, which is frequently associated with synthetic insecticide up use in plant protection.(Mokhena *et al*., 2016). Neem is well known in Northern part of Nigeria, where for many years it is one of the most versatile medicinal plants, having a wide spectrum of antimicrobial activity (Alves *et al*., 2009; Atawodi and Atawodi, 2009). *Azadirachtin* is the most active compound of neem, which is toxic for insects(Sarkar *et al*., 2021). *Azadirachtin* is mostly extracted from compressed neem seeds, thus used as pest repellents. These compounds act on the basis of interfering with insects' endocrine system (Anibal, 2007).

Some limonoid compounds with pesticidal properties have been extracted from neem seeds and shown to inhibited pest growth and the most effective among this compound is Azadirachtin (Alves *et al*., 2009). *Azadirachtin* stimulate specific deterrent cells in chemoreceptors and also blocks the firing of sugar receptor cell which normally stimulate feeding. Other effect of *Azadirachtin* is physically affecting the growth and development of insect while physiological effect could be directly affecting the cell tissues or indirectly affecting the endocrine system of the insects.(Luntz) & Nisbet, 2000). Weeding can increase yield and calyx size but may also reduce profit for the farmer. Now that the calyx yield is considered as important as the ribbon yield of Roselle, there is a need to understand the cheapest and affordable way of pest control using NLAE to minimize the cost of production. Neem-based insecticides are known for their pesticidal activity against so many 400 species of insects (Siddiqui *et al.,* 2003). These insecticides have been advocated to replace synthetic insecticides as they are more sensible to be used in most pest management programs (Irigaray *et al.,* 2010). Thus, neem extract pesticides are available for use against many pests which currently under further investigation as an alternative to synthetic pesticides (Anibal, 2007). Hence, the need to evaluate the effect of different graded concentration of Neem leaves extract against *Podagrica spp* population affecting Roselle.

MATERIALS AND METHODS

A field experiment was conducted in the rainy season of 2020 at the Teaching and Research Farm of the Kebbi State University of Science and Technology in Aliero and Jega town Orchards, respectively. The treatment consisted of three varieties of Roselle (V1 as green variety, V2 as red variety, V3 as deep red variety) and three graded level of NLAE (C1-1000mg/mL, C2-500mg/mL, C3-333mg/mL) and control (C0-0mg/mL), laid out in randomized complete block design (RCBD) in a split plot arrangement and replicated three times. Roselle varieties variable were assigned to the main plots, while the NLAE concentrations variables were assigned to the subplot. Plots of 3.0 m x 3.0 m (9.0 m^2) with 0.5 m between plots were prepared. Ripe neem leaves were collected and de-pulped, washed and dried under the shade. Seeds were weighed according the respective concentrations (x g; u g and y g for 10% 20% and 30% respectively). The weighed leaves were ground separately using mortar and pestle, soaked in 2.0 litres of water, left overnight then filtered the following day using a muslin cloth and sprayed accordingly on Roselle. Roselles were sprayed with neem extract and water added as an adjuvant and mixed thoroughly before spraying on the leaves of the Roselle plots when the plant reached stage of 50% flowering for the control of *Podagrica spp*. Data collected were subjected to analysis of variance as described by Snedecor and Cochran (1967) using SAS software (SAS Institute, 1999). The treatment means were compared using Duncan Multiple Range Test (Duncan, 1955).

RESULTS

Effects of Neem Leaf Aqueous Extract (NLAE) on *Podagrica spp* Population

The result in Table 1 revealed that, there was no significant difference ($P<0.05$) among treatments on the population of the *Podagrica spps* at one (DBS) and 1 DAS. However, at 2 DAS the result showed that untreated recorded the highest number of *Podagrica* spp (22.39), although not significantly different from 2nd (21.94) and 3rd (21.89) concentrations. Concentration 1 recorded the lowest *Podagrica* spp population (12.28) and same goes with 3 DAS. However, at 5 DAS, the result revealed that the untreated control recorded the highest number of *Podagrica* spp (12.28), and was significantly different from other treatments while all other concentration of NLAE did not significantly differ. At 7 DAS, the untreated also had the highest number of Po*dagrica* spp although it was not significantly different from concentration 2, but concentration 1 recorded the lowest population of *Podagrica* spp and was significantly different from other treatments.

The varietal effect of NLAE on the population of *Podagrica* spp on roselle plant revealed that, the green variety had the highest population (38.50) of *Podagrica* spp at 1 DBS and was significantly different ($P < 0.05$) from the others while the red variety had the least population.

At 1 DAS there was significant difference among treatments. The green variety had the highest number of *Podagrica* spp (31.13) and this was significantly different from other treatments. While the red variety had the least population (25.04) of *Podagrica* spp. Similarly, at 2 DAS also, the green variety recorded the highest number of *Podagrica* spp (23.67) although it was not significantly different from the deep red variety and the red variety had significantly lower population (18.50) of *Podagrica* spp. At 3 DAS the result was same as 2 DAS Whereas, at 5 DAS there was no significance difference among treatments. At 7 DAS, the green variety also recorded the highest number (5.67) of *Podagrica* spp with no significance difference from deep red variety while the red variety recorded the least (3.75). Meanwhile, there was no significant interaction between the NLAE's concentration levels and the varieties on *Podagrica* spp among all the treatment.

Table 1: Effects of NLAE on Population of *Podagrica spp* on Roselle Plant in Aliero

Treatment	1 DBS	Days after spray (DAS)				
		1	2	3	5	7
Conc. of NLAE (kg/ltr)						
0 0mg/mL	34.00[a]	28.50[a]	22.39[a]	22.39[a]	12.28[a]	6.33[a]
C1-1000mg/mL	32.78[b]	26.83[b]	18.28[c]	18.28[c]	6.78[d]	3.44[e]
C2-500mg/mL	34.00	27.39	21.94[a]	21.94[a]	9.06[b]	5.17[ab]
C3-333mg/mL	33.00	28.11	21.89[a]	21.89[a]	8.78[b]	4.22[b]
SE ±	1.17	1.36	1.23	1.23	0.81	0.43
Variety						
Green	38.50[a]	31.13[a]	23.67[a]	23.67[a]	10.92	5.67[a]
Red	29.08[c]	25.04[c]	18.50[b]	18.50[c]	7.79	3.75[b]
Deep Red	33.13[b]	26.96[c]	21.21[b]	21.21[ab]	8.96	4.96[a]
SE±	1.01	1.17	1.07	1.07	0.69	0.34
Interaction						

Means with the same letter are not significantly different at 5% probability level according to Duncan multiple range test. 1 DBS stands for a day before spraying of treatment, where no letter exist differences were not significant.

The result on Table 2 revealed that at one DBS, there was no significant difference ($P < 0.05$) among treatments on the population of the insect pest. But at 1 DAS, there was no significant difference between the untreated control and the treated plots, however there are no significant difference among the treated plots. However, at 2 DAS the result showed that there was significant difference ($P< 0.05$) among the treatments, the untreated control also recorded the highest number of *Podagrica spp* (32.44), concentration 1 recorded the lowest *Podagrica spp* population (19.67) while there was no significant difference between concentration 2 and 3 At 3 DAS same trend was at 1DAS was observed, with the untreated control sustaining significant higher population number *Podagrica* spp and no significant difference among the NLAA concentration. At 5 DAS, the result also revealed that untreated control recorded the highest number of *Podagrica spp* (18.89), and was significantly different among the treatment while concentration 1 had the least number of *Podagrica spp* (5.22) followed by concentration 2 and 3. At 7 DAS, there was significant difference among the treatments. The untreated control also had the highest number (16.11) of *Podagrica spp* and concentration 1 recorded the lowest population (3.78) of *Podagrica spp*.

The varietal effect of NLAE on the population of *Podagrica spp* on roselle plant revealed that, the green variety had the highest population (40.44) of *Podagrica spp* at 1 DBS and was significantly different from the others while the red had the least population (35.42). At 1 DAS there was significant difference among treatment also where the green variety had the highest number of *Podagrica spp* (37.75) while the red variety had the least population (28.91) of *Podagrica spp*, but not significantly different from the deep red variety. At 2 DAS also, the green variety recorded the highest number of *Podagrica spp* (30.08) which was significantly different from the deep red variety and the red variety had the least population (22.42) of *Podagrica spp*. At 3 DAS also, the green variety recorded the highest number of *Podagrica spp* (20.30) which was significantly different from the deep red variety and the red variety which had the least population (16.58) of *Podagrica spp*. Likewise, at 5 DAS there was significant difference among treatments. The green variety had the highest number of *Podagrica spp* (13.00) which was significantly different from the deep red variety and the red variety recorded the least population (8.08) of *Podagrica spp.* At 7 DAS, the green variety recorded the highest number (10.58) of *Podagrica spp* and was significantly different from deep red variety and red variety that had the least

(7.25). However, there is a trend of dynamics of steady declination of the population from 1DBS to 7DAS

Table 2 Effects of NLAE on Population of *Podagrica Spp* on Roselle Plant in Jega

Treatment	1 DBS	Days after spray (DAS)				
	0	1	2	3	5	7
Conc. of NLAE (kg/ltr)						
0 0mg/mL	40.44[a]	36.67[a]	32.44[a]	24.33[a]	18.89[a]	16.11[a]
C1-1000mg/mL	38.78[b]	29.11[c]	19.67[c]	14.00[d]	5.22[c]	3.78[c]
C2-500mg/mL	38.33[b]	30.56[b]	23.22[c]	15.78[c]	7.33[b]	4.22[c]
C3-333mg/mL	37.56[c]	31.56[b]	25.00[b]	18.44[b]	7.78[b]	9.89[b]
SE±	0.94	0.94	0.99	1.94	0.95	1.09
Variety						
Green	44.92[a]	37.75[a]	30.08[a]	20.30	13.00[a]	10.58[a]
Red	35.42[b]	28.91[b]	22.42[b]	16.58	8.08[b]	7.25[b]
Deep Red	36.00[b]	29.25[b]	22.75[b]	17.33	8.33[b]	7.67[b]
SE±	0.82	0.81	0.86	1.68	0.82	0.94
Interaction						
C×V	NS	NS	NS	NS	NS	NS

Means with the same letter are not significantly different at 5% probability level according to Duncan multiple range test. 1 DBS stands for a day before spraying of treatment, where no letter exist differences were not significant.

DISCUSSION

The findings obtained indicated that neem leaf extract is exhibited some degree of insecticidal activity in reducing flea beetle population on Roselle in the field. Both 1000mg/mL and 500mg/mL conferred high levels of protection to Roselle in reducing flea beetle population on Roselle, the untreated control (0mg/mL) suffered significant (P>0.05) beetle infestations than treated plots. This agrees with the findings of Olaniran et al. (2013) who stated that plant extracts from especially from Neem had potent insecticidal properties against insect on vegetables. The sever reduction in the pest count on Roselle treated with 500mg/mL of Neem leave extract leaves similar to that reported by Amugi et al. (2012) who reported that plant extracts conferred similar protection to okra with synthetic insecticide Lambdacyhalothrin in the control of flea beetles. The result obtained in the untreated plot is corroborated with the findings of Parh et al., (1997) that significantly, higher beetle infestation was recorded in the untreated okra plot to those of the Neem leave plots plots. Schmutterer (1990), who stated that plants contain highly insecticidal properties against insect pests of crops by providing a rich source of biologically potent chemical compounds which can be used as safer insecticides.

Findings from the field of study to determine the *Podagrica spp* as a major pest complex affecting Roselle production in Aliero, Kebbi State The present findings corraborates that of Fasunwon and Banjo (2010) who reported that among the insect pests attacking Roselle plant, *Podagrica spp.* cause economic damage, attacking the lamina of the young foliage and matured leaves which result in reduction of the photosynthetic ability of the crop. Komolafe (1979) observed that *Podagrica species* did not only damage the leaves and flowers but caused premature falling of the pods. This insect also transmits mosaic virus resulting in 20-50% yield reduction (Fajinomi and Fajinmi, 2006). *Zonocerus variegatus* feed on wide range of crops including roselle (Babatunde and Akinsete, 2001).

Experimental trials in Aliero, Kebbi State revealed that the efficacy of the NLAEs at different concentration levels on pest affecting Roselle, and it showed that NLAE at untreated control had the highest number of *Podagrica spp* from day 1 to day 7, while concentration 1 recorded the least number of *Podagrica spp* followed by 2 and 3 to untreated control respectively.

The result from the study showed that in Jega location, *Podagrica spp,* are the major insect of roselle at flowering stage. The percentage loss of leaves, calyces and seeds due to damage by the *Podagrica spp* flea

beetles has not been reported partially due to report by some researchers that insect pest damage is a minor problem in roselle. (Erichsen and Schoeman, 1993). Also, *Podagrica spp* fed voraciously on the lamina and young leaves of the Roselle plant. The study corroborates the findings of Fasunwon and Banjo (2010) who reported that among the insect pests attacking Roselle plant, *Podagrica spp.* cause economic damage, attacking the lamina of the young foliage and matured leaves which result in reduction of the photosynthetic ability of the crop.

In Jega location, the efficacy of the NLAEs at different concentration levels on *Podagrica spp* flea beetles affecting Roselle, indicated that the higher the concentration level, the lower the number of *Podagrica spp*, from day 1 to day 7. Kabaru and Gichia (2001) argued that botanical insecticide is a promising alternative in the protection of crops against insect pests. They are generally pest-specific and relatively harmless to non-target organisms. *Azardirachtin* derived from the Neem tree (*Azadirachta indica L.*) has been reported as antifeedant, repellent, oviposition deterrent and insect growth regulator (Isman, 2008; Naumann and Isman, 1996; Prijono and Hassan, 1993). This evergreen, fast-growing plant known as the Indian lilac offers immense antifeedant properties due to its efficacy in suppressing the feeding sensation in insects, at concentrations even less than 1 parts per million (Isman *et al.*, 1990)

CONCLUSION

This research reflected that neem leave extract has significantly impact on reducing infestation and population of *Podagrica spp* beetles on Roselle in both experimental spaces used. Therefore, neem leave extract could serve as potential alternative insecticides control on Roselle. It is recommended that the development of standardized formulation and certification of the Neem-pesticide products is even more crucial and highly recommended.

REFERENCES

Abiodun, O. O., Olaitan, A. F., Yetunde, Y. S., & Abiodun, A. T. (2018). Bioefficacy of Petiveria alliacea and Annona squamosa against Podagrica species of Roselle (Hibiscus sabdariffa Linn). *Journal of Entomology and Zoology Studies*, *6*(4), 862–866.

Alves P. D., Brandão M. G. L., Nunan E.A. and Vianna-Soares, C.D. (2009). Chromatograhic Evaluation and Antimicrobial activity of Neem (Azadirachta indica A. Juss., Meliaceae) leaves hydro-alcoholic extracts. *Brazilian Journal of Pharmacology*, 19:510-515.

Amugi, C. F., Echezona, B. C. and Dialohe, S. A. (2012). Extraction fractions of ginger (Zingiber offinale F.) and residue in the control of field and storage pests. *Journal of Agriculture and Veterinary Science.* 4: 45-52

Anibal F, and Condor, G. (2007). Effect of neem (Azadirachta indica A. Juss) insecticides on parasitoids. *The Peruvian Journal of Biology*, 14: 69-74

Anjah, G. M O. Y. Ogunsanwo, S. O. Jimoh, J. N. Farjoh and F. M. Tsombow, (2012). Assessment of regeneration potential of *Hibiscus sabdariffa L*, under established ecosystem in Cameroon. *Journal of Horticulture and Forestry*. 4(6), pp. 96-102.

Ansari, M. (2013). An Overview of the Roselle Plant with Particular Reference to Its Cultivation, Diseases and Usages. *European Journal of Medicinal Plants*, *3*(1), 135–145. https://doi.org/10.9734/ejmp/2013/1889

Atawodi S.E. and Atawodi J.C. (2009). Azadirachta indica (neem): a plant of multiple biological and pharmacological activities. *Phytochemistry Reviews*, 8: 601-620.

Babatunde I, A. and Akinsete, A. (2001). The attraction of Zonocerus variegatus (Orthoptera: Pyrgomorphidae) to different types of lure. *Revista de biologia tropical*, 49, pp 673-678.

Duncan, D. B. (1955). "Multiple range and multiple F tests". *Biometrics.* 11: 1–42.

Erichsen, C. and Schoeman, A.S. (1993). *Monoleptaapicalis* SAHLBERG (Coleoptera:Chrysomelidae): A New Addition to the Avocado Pest Complex.*South African Avocado Growers' Association Yearbook*. 16:123-124.

Fajinmi, A.A. & Fajinmi, O.B. (2006) Incidence of okra mosaic virus at different growth srages of okra plants (Abelmoschus esculentus L. Moench) under tropical condition. *Journal of General and Molecular Virology*, 2, pp 028-031.

Fasunwon, B.T. and Banjo, A.D. (2010) Seasonal population fluctuations of Podagrica Species on okra plant (*Abelmoschus esculentus*). *Res.J.Agric.Biolog.Sci*, 6, pp 283-288.

Futuless, K.N., Kwaga, Y., and Clement, T. (2010). Effect of sowing date on the Calyx yield and Yield components of roselle (*Hibiscus sabdariffa* L.) in Northern Guinea Savanna. *New York Science Journal*, 3 (11), pp 1-4.

Irigaray F., Javier S.D.C., Fernando M.G., Vicente M. and Ignacio P.M. (2010). Acute and

Reproductive Effects of Align, an Insecticide containing Azadirachtin, on the Grape Berry Moth, (Lobesia botrana), *Journal of Insect Science,* 10: 1-11

Isman MB (2008) Botanical insecticides: for richer, for poorer. Pest Manag Sci 64:8–11.

Isman, M.B., O. Koul, A. Luczynski and J. Kaminski, (1990). Insecticidal and antifeedant bioactivities of neem oils and their relationship to azadirachtin content. *J. Agric. Food Chem.*, 38: 1406-1411

Isman, MB and Naumann K, (1996). Toxicity of neem (*Azadirachta indica* A. uss) seed aqueous to larval honeybees and estimation of dangers from eld applications. *Am Bee J* 136:518–520

Kabaru, J.M. and Gichia, L. (2001) Insecticidal activity of aqueous derived from different parts of the mangrove tree *Rhizophora mucronata* (Rhizophoraceae) Lam. against three arthropods. *African Journal of Science and Technology*, 2 (2).

Komolafe, M.F. (1979). Agricultural Sciences for most West Africa Schools and Colleges. University of Ibadan press Ltd: Ibadan, Nigeria.

Mordue(Luntz), A. J., & Nisbet, A. J. (2000). Azadirachtin from the neem tree Azadirachta indica: its action against insects. *Anais Da Sociedade Entomológica Do Brasil*, *29*(4), 615–632.

Mokhena, T., Mochane, M., Tshwafo, M., Linganiso, L., Thekisoe, O., & Songca, S. (2016). We are IntechOpen , the world ' s leading publisher of Open Access books Built by scientists , for scientists TOP 1 %. *Intech*, 225–240.

National Research Council (1992). Neem: a tree for solving global problems, report of an ad-hoc panel of the Board on Science and Technology for International Development National Academy Press, Washington, DC.

Olaniran, O. O., Owosina, Osai F, O. (1982). Chemical control of flea beetles (Podagrica spp.) on okra, The 25th Annual Conference of the Horticultural Society of Nigeria, Zaria. Research, Life Sciences. 1968: 17: 150-154

Parh, I, A, Mgbema M. O., Anozie O. C., Tanyimboh, E. N. (1997). Incidence and control of insect pests of vegetable crops on Jos Plateau, *Nigerian Journal of Entomology*, 4:7- 22.

Prijono. D, Hassan, E. (1993). Laboratory and field efficacy of neem (*Azadirachta indica* A. Juss) aqueous against two broccoli pests. *Zeitschrift fur P. antzenkrankheiten und P. Antzenschutz*, 100; 354-70.

Robert, L. (2008). Insect Control in Ullman's Encyclopaedia of Industrial Chemistry. Wiley-VCH, Weinheim, 263 pp. Schmutterer H. (1990). Properties and potential of natural pesticides from the neem tree, Azadirachta indica. Annual Review Journal of Entomology, 35: 271-297

SAS Institute Inc., SAS/C (1999). OnlineDoc®, Release 6.50, Cary, NC: SAS Institute Inc.,

Sarkar, S., Singh, R. P., & Bhattacharya, G. (2021). Exploring the role of Azadirachta indica (neem) and its active compounds in the regulation of biological pathways: an update on molecular approach. *3 Biotech*, *11*(4), 1–12.

Schippers, R.R. (2000). African Indigenous Vegetables. An overview of the Cultivated Species. Natural Resources Institute, Chatham, UK Natural Resources Institute/ACP-EU Technical Centre for Agricultural and Rural Cooperation. Pp 119-133.

Schmutterer H. (1990). Properties and potential of natural pesticides from the neem tree, Azadirachta indica. *Annual Review Journal of Entomology*, 35: 271-297.

Siddiqui BS, Afshan F, Gulzar T, Sultana R, Naqvi SNH, and Tariq RM. (2003). Tetracyclic triterpenoids from the leaves of *Azadirachta indica* and their insecticidal activities. *Chemical and Pharmaceutical Bulletin* 51:415–417.

Snedecor, G. W. and Cochran, W. G. (1967). Statistical Methods. 6th edition. Iowa State University Press, Ames Iowa U.S.A. 456pp.