Doña Lawa-an H. Lopez Campus Iloilo City

SPERMICIDAL EFFECT OF NEEM (Azadirachta indica) EXRTACTS

A Research Paper Presented to the
Faculty of the Philippine Science High School Western Visayas

Iloilo City

in Partial Fulfillment
of the Requirements in
Technology Research II

by

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March 2001



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APPROVAL SHEET

A Research Paper Requirement

For Technology Research II

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Abstract

This study determined whether or not neem (Azadirachta inidca) extracts had an effect on sperm mortality. It was hypothesized that there was o significant difference between the 0%, 5%, 10%, and 15% concentrations of neem extract spermicide on the mortality of sperm. Statistical tools employed in the study were the standard deviation and mean as descriptive statistical tools, and One-Way ANOVA and Scheffe test as inferential statistical tools.

Results showed that neem extracts had a significant effect on sperm mortality at 10% and 15% concentrations and that there was no significant difference between the effectiveness of the 10% and 15% concentrations of neem spermicide.

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SPERMICIDAL EFFECT OF NEEM (Azadirachta indica) EXTRACTS

Chapter 1

Introduction to the Study

Background of the Study

Due to financial limitations, most Filipino families could not afford a large household. Yet sexual urges in many are hard to control. This is where family planning comes in. Some methods used to avoid unwanted pregnancies are rhythm method, coitus unterruptus (withdrawal), sterilization, and other contraceptive measures, among which, is the use of spermicides.

No contraceptive has yet been found to be 100 percent reliable, suitable, practical, and without side effects or possible health risks. A greater understanding of reproductive anatomy and physiology leads to the wide spread use of a number of methods and devices of varying reliability and risk.

Sterilization, after abstinence is the most effective form of contraception, is permanent and generally irreversible. But, it only works for those who are satisfied with having completed their families. Abstinence, on the other hand is out of the



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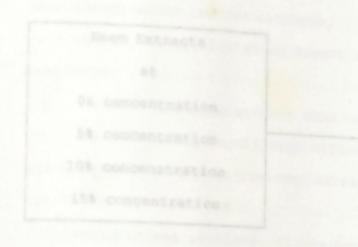
question, you'd have to be a saint to suppress those desires when aroused, since, our world is filled with sexually active people. Rhythm method and coitus unterruptus are unreliable, unpredictable, and least effective. Coitus interruptus could upset the sexual partner especially when in the consummation of lovemaking. Most of the contraceptive methods are either unreliable or permanent, which leaves us with spermicides. Spermicides with the help of other contraceptive devices are effective up to 97% with careful use. It is temporary, easy to use, and protects against sexually transmitted diseases. Spermicides are quite convenient for they can be used at any age. Although there are risks and failures associated with the use of contraceptives, it should be emphasized that, with careful use, most of them work safely most of the time.

Figure 1 shows how sperm count is affected by the different concentrations of neem seed extracts. The independent variables are the treatments namely; neem extracts, neem extracts with distilled water, neem extracts with distilled water and sodium bicarbonate (NaHCO3), and neem extracts with formalin, sodium

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bicarbonate (NaHCO3), and distilled water. The dependent variable is the sperm count



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INDEPENDENT VARIABLE DEPENDENT VARIABLE

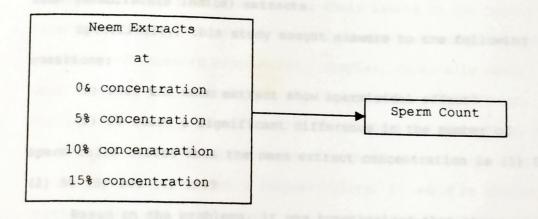


Figure 1. The effect of various concentrations of neem on sperm count.

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Statement of the Problem and Hypothesis

This study aimed to determine the spermicidal effect of neem (Azadirachta indica) extracts.

Specifically, this study sought answers to the following questions:

- (1) Does the neem extract show spermicidal effect?
- (2) Is there a significant difference in the number of sperm cells killed when the neem extract concentration is (1) 0% (2) 5% (3) 10% (4) 15%?

Based on the problems, it was hypothesized that there would be no significant difference in the number of sperm cells killed when the neem extract concentration is (1) 0%, (2) 5%, (3) 10%, (4) 15%.

Significance of the Study

A large percentage of sexually active persons use contraceptives to avoid transmission of diseases and unwanted pregnancies. Especially the financially unstable, which many of our countrymen are, they could not afford the complications of a large family. The more their kids, the bigger their problems,

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they could hardly support themselves, now they'd have more mouths to feed. With contraceptives such as spermicides, possibility of conception would be greatly diminished. Couples, who are unprepared to have children because their assets do not permit them yet, could still perform their intimate lovemaking without the worry of unwanted pregnancies. Couples, especially newly weds, need to prepare and plan for their families. It is the obligation of every parent to give the child a bright future, to be able to support and provide for the child. But if the couple is not yet ready for such a responsibility, it would be unwise to continue to populate the human race. Spermicides and other contraceptives are a preventive measure for those who do not wish to conceive and populate the Earth.

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Definition of Terms

For the purpose of clarity, the following terms are being defined:

Spermicide- is a chemical substance that that immobilizes sperm (Wagman, 1993).

In this study, it would mean the neem extract applied on the sperm samples.

Neem- is a tree whose extracts are used as an effective spermicide (www.neemaura.com/, 1999).

In this study, it would mean the fruit of a neem plant extracted to be used as a spermicide.

Extract- is a substance or preparation obtained by distillation or other chemical means (The Webster Handy College Dictionary, 1993).

In this study, the term is referred to as the liquid or juice obtained from the distillation of the neem seeds.

Effect- means result or outcome (Collins Pocket Dictionary and Thesaurus, 1993).

In this study, it would mean the result after neem extracts were applied on the sperm.

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Concentration- means dilution, strength, or intensity (Collins Pocket Dictionary and Thesaurus).

In this study, it would mean the different amount of neem extracts applied on the sperm.

Time - duration, interval, span (Collins Pocket Dictionary and Thesaurus).

In this study, it would mean the duration of optimum effectivity of the neem extracts as a spermicide.

Sperm Count - determines the number of sperm present in a single ejaculation (Wagman, 1993).

In this study, it would mean the number of sperm determined before and after the neem extracts are applied.

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Scope and Delimitation

This study aimed to test Neem extracts for their spermicidal effect. There were three replicates for each sperm sample. The statistical tools employed were the mean standard deviation, One-Way ANOVA, and the Scheffe test. It was not able to test its effectiveness with respect to time factors and its effectiveness was not compared to the effectiveness of commercial spermicides.

This study was conducted from April 19, 2000 to December 12, 2000 at the Divine Word College and Philippine Science High School Research Laboratory.

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Chapter 2

Review of Related Literature

Upon the unfolding of the twenty-first century, mankind welcomed the idea of respecting the ecosystem in relation with population size. People have realized that due to persisting expansion, countless species of flora and fauna were forced to extinction.

Innumerable ways of controlling and inhibiting the unmanageable population were studied. Habits and processes were developed to promote a breathable society. Among these was the discovery and use of spermicides. Its benefits to humanity have long ago been realized but only artificially. We need to develop one that would benefit us as well as the environment, one that is natural and biodegradable.

To the Indians, this was no problem

(www.neemfoundation.com;1998). They have harnessed the spermicidal powers of a most reputable plant, the neem

(Azadirachta indica) plant. It not only provided them with birth control but also other attributes like insecticidal medical properties (www.neemfoundation.com). It is only recently that the world has laid its eyes on this botanical plant.

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During the later years of the last century, Indian scientists have pioneered exposing to the world the reputable qualities of this plant. It was they who first developed the neem spermicide (www.neemfoundation.com;1998).

Lately, foreign scientists have realized the potential properties of this plant to prohibit pregnancy. They too played accomplice in the promotion of the natural spermicide. In a test of neem's birth control effects with members of the Indian Army, daily oral doses of several drops of neem seed oil in gelatin capsules were given to twenty married soldiers. The effect took six weeks to become 100 percent effective; it remained effective during the entire year of the trial and was reversed six weeks after the subjects stopped taking the capsules. During this time the men experienced no adverse side effects and retained their normal capabilities and desires. There were no pregnancies of any of the wives during the period of the study (www.neemaura.com;1999).

The studies leading to the development of these products proved that neem oil killed sperm in the vagina within thirty seconds and was effective for up to five hours

(www.neemaura/UsesPages/birthcon.htm;1999).

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Chapter 3

Methodology

Purpose of the Study and Research Design

This study aimed to determine the spermicidal effect of neem (Azadirachta indica) extracts.

Specifically, this study determined if neem extract shows spermicidal effect. It also determined the significant difference in the number of sperm killed when the neem extract concentration is at 0%, 5%, 10%, and 15%.

Based on the problems, it was hypothesized that there is no significant difference on the number of sperm killed when the neem extract concentration is at 0%, 5%, 10%, and 15%.

Research Design

During the conduct of the study, the researchers followed the pre-test post-test research design. The sperm counting of the five seminal fluid samples was pre-tested. After which, the treatment was applied, then a post-test sperm count was conducted.

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The inferential statistical tool employed in the conduct of the study is the one-way ANOVA, with Scheffe, since there are five categories of variables given in the study.

Materials

In testing the spermicidal properties of Neem extracts, the researchers needed a 50-mL Erlenmeyer Flask, a 250-mL Distilling Flask, a condenser, rubber tubing, iron stands, iron rings, an adapter, a hot plate, wire gauze, a microscope, a Newbauer counting chamber, 50-mL beakers, marking pens, a graduated cylinder, glass slides, glass slips, rubber stoppers, live sperm cells, neem seeds, formalin, sodium bicarbonate (NaHCO3), and distilled water.

Methods

Gathering and Preparation of Neem Seeds

The researchers first obtained Neem seeds from various trees located in the Philippine Science High School Campus. These were weighed until a kilogram. The seeds were then placed in a blender and grinded until texture is fine and smooth.

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Distillation

The researchers prepared the Neem seeds for extraction. They placed the seeds in the distilling flask to be distilled in groups of 250 mL. The extracts were collected in an Erlenmeyer flask and kept in a cool dry place.

Canvassing for Donors

The researchers sought at least five possible donors for who are willing to donate their sperm to the researchers. They agreed on an exact date and time the sperm samples are to be delivered to the researchers to prevent unnecessary mortality among the sperm.

Treatment Application

The researchers treated the sperm at different concentrations of Neem extracts only, Neem extracts with distilled water, Neem extracts with distilled water and Sodium Bicarbonate (NaHCO3), and Neem extracts with distilled water, Sodium Bicarbonate (NaHCO3), and Formalin.

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Sperm Count

The sperm count is performed by diluting the sample with the spermicide and counting the number of living sperm cells in the Neubauer chamber. This was done by diluting the sample with the solution in a 1:20 ratio. The sperm are counted in five RBC squares (see Figure 2). The number of sperm counted is then multiplied by one million to calculate the number of sperm per milliliter. See example below.

- A. 60 sperm counted X 1,000,000 = 60,000,000 sperm/mL
- B. 60 sperm X 20 (dilution) = 60,000 5 (squares counted) X 0.004µL (volume counted) sperm/µL

Data Gathering

The researchers then analyzed the sperm samples. The sperm mortality was taken to account and recorded. Per concentration, the mean was computed and their differences were subjected to testing using the One-Way ANOVA for any significance. The average mean of the sperm mortality was compared to the mean of a commercial spermicide using the t-test for any significance.

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Statistical Data Analysis Procedure

Certain statistical tools will be used in this study. The mean and standard deviation will be used as descriptive statistical tools, while the One-way Analysis of Variance (ANOVA), and the Scheffe test will be used as inferential statistical tools.

Descriptive Statistical Tools

Mean. The mean will determine the average sperm mortality.

Standard Deviation. The standard deviation will determine the closeness of the obtained values to their means.

Inferential Statistical Tools

One-Way Analysis of Variance (ANOVA). The One-Way ANOVA, set at .05 alpha level of significance will determine the significant difference in the mortality of the sperm among the different concentrations of the Neem seed extract solution.

Scheffe Test. The Scheffe Test, also set at .05 alpha level of significance will be used as a post-hoc multiple comparison test following the significant difference in the One-Way ANOVA test.

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Chapter 4

Results

This study aimed to determine the:

Spermicidal effect of neem (Azadiracta indica) extracts. It also determined the difference in effectiveness of various concentrations of the neem at 0%, 5%, 10%, and 20%.

It was hypothesized that there is no significant difference in the number of sperm cells killed when the neem extract concentration was 0%, 5%, 10%, and 15%.

Mortality of Sperm Cells at Different Concentrations of Neem Extract

Several of the Sperm cells died with the treatment of Neem exrtact spermicide.

The mean mortality at the different concentrations were 6.33, 54.00, 108.33, and 162.33 for 0% concentration, 5% concentration, 10% concentration, and 15% concentration respectively.

Table 1 shows the data.



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Table 1
Means of Sperm Count

Category	N	Pretest Mean	Std. Deviation	Postest Mean	Std. Deviation	Dead Cells	Std. Deviation
0%	3	274.00	39.69	267.67	42.72	6.33	3.21
5%	3	301.00	26.63	247.00	43.55	54.00	17.44
10%	3	269.67	51.64	161.33	79.48	108.3	34.30
15%	3	278.33	49.90	116.00	2.00	162.3	51.73
Total	12	280.75	38.89	198.00	77.49	82.75	66.94

Differences in the Mortality of Sperm Cells at Different Concentrations of Neem Extract

The One-Way ANOVA showed that there was a difference of the Sperm Cells treated with different concentrations of Neem Extract Spermicide, as reflected by $\underline{F}=0.002$, $\underline{P}<0.05$.

Table 2 shows the data.

The Scheffe Test showed the significance in the mean mortality of the sperm cells existed among 10% and 15% concentrations.

Table 3 shows the data.

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One-Way ANOVA of the Number of Dead Cells treated with Different
Concentrations of Neem Extract

100	is study at	Sum of Squares	df	Mean Square	F	Sig.
DIFF	Between Groups	40962.25	3	13654.08	13.107	0.002
	Within Groups	8334.000	8	1041.750	Ebot 1	
	Total	49296.25	11	contract in the	and a second	

Table 3
Scheffe Test of One-Way ANOVA in Table 2

Category		Mean Difference	Standard Error	Sig.
0.00	5.00 10.00 15.00	-47.67 -102.00* -156.00*	26.353 26.353 26.353	0.407 0.031 0.003
5.00	10.00 15.00	-54.33 -108.33*	26.353 26.353	0.307
10.00	15.00	-54.00	26.353	0.312

^{*.} The mean difference is significant at the .05 level.

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Chapter 5

Summary, Findings, Conclusions, Recommendations

Summary

This study aimed to test neem (Azadiracta indica) extracts at different concentrations for its spermicidal effect.

Specifically, this study answered the following questions:

- 1. Does the neem extract show spermicidal effect?
- 2. Is there a significant difference in the number of sperm killed when the neem extract concentration is (1) 0% (2) 5% (3) 10% (4) 15%?

Based on these problems, it was hypothesized that there exists a significant difference in the:

Effectiveness of the neem extract spemicide at 0%, 5%,
 and 15% concentration.

Findings

This study was able to establish the following findings based on the data gathered:

Neem is an effective spermicide at 10% and 15%
 concentrations.

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2. There is a significant difference in the effectiveness of the neem extract spermicide at various concentrations, namely 10% and 15% concentrations.

Conclusion

Based on the results of the study, the following can be concluded:

Neem Extracts have the potential of becoming an effective spermicide as shown by the mean mortality of 6.33, 54.00, 108.33, and 162.33 per sample. There was a significant difference in the effectiveness of the neem extract spermicide at different concentrations. Only concentrations, 10% and 15%, therefore are as potent as each other.

Recommendations

It is recommended that interested researchers continue and pursue this study and its other areas.

Future studies can include time exposure as a factor that can affect the mortality rate of the sperm.

Also, the researchers recommend that future researches be conducted on other plants that may have effective spermicidal properties.

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References

Collins Pocket Dictionary and Thesaurus. (1993). Great Britain:
Harper Collins Publishers.

The Webster Handy College Dictionary. (1993). New York: Black Dog and Levinthal Publishers, Incorporated.

Wagman, R. (1993). The New Complete Medical and Health

Encyclopedia. Chicago: J.G. Ferguson Publishing Company.

www.neemaura.com; (1999)

www.neemaura/UsesPages/birthcon.htm; (1999)

www.neemfoundation.com; (1998)

