**IMPACT: International Journal of Research in Applied, Natural and Social Sciences (IMPACT: IJRANSS)**
**ISSN (P): 2347-4580; ISSN (E): 2321-8851**
**Vol. 6, Issue 7, Jul 2018, 19-26**


# EFFECT OF TWO PLANT EXTRACTS, *AZADIRACHTA INDICA* A. JUGS AND *OCIMUM SANCTUM* L.ON MORPHOHISTOLOGY OF REPRODUCTIVE ORGANS OF *ODONTOPUS NIGRICORNIS* STAL

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**Received:** 21 Jun 2018 **Accepted:** 28 Jun 2018 **Published:** 12 Jul 2018

## *ABSTRACT*

*Effect of two test plants **A. indica** and **O. sanctum** is observed on the reproductive organs of **O. nigricornis** 2 percent concentration of the extract of both the test plants showed significance morphological suppressive effect on the shape size, weight and growth of both ovaries and tests of adult bugs in comparison to control bugs disruptive effect of the extract of **A. indica** on the histology of ovaries is seen comparatively much severe than that treated with **O. sanctum**. Similarly, effect of the extract of **A. indica** on the histology of testes of male bugs was more pronounced and suppressive than the treated with **O. sanctum**. Various histological deformities are seen in ovaries and testes of the bugs treated with both the test plants extracts.*

***KEYWORDS:*** *O. Nigricornis, O. Sanctum, Histological Deformities*

## INTRODUCTION

The use of synthetic insecticides dominates the scene of crop production due to their, many fold advantage. But, their extensive application during the past decades has caused environmental pollution, health hazards, associated with toxic, residue, and development of resistance by the pest and pest resurgence (Joshi, 1986). This necessitated the need of development of alternate and eco-friendly pest protection technologies. As a result, in recent years botanical insecticides from plant products or extracts have attracted the attention world over. In view of this two plant extracts of Neem, ***Azadirachta indica*** and ***Ocimum sanctum*** are tested on their effect on the reproductive organs of ***Odontopus nigricornis*** Stal (Heteroptera-Pyrrhocoridae) a pest ***Pterygota alata*** Roxb. (Sterculiaceae). A deep peep into the review of literature revealed that some entomologists have made historical records by examining the inhibitory biological effect of a good number of test plants against insect pests particularly concerned with morphohistology of Reproductive organs. Among these main contributors are –Koul (1984), Srivastava (1993), Verma (2004), Mohmmad ***et.al.*** (2010) and Sharaley ***et.al.*** (2011). In the present study, an endeavor has been made to note the effect of aforesaid two test plant extracts on the morphology and histology of male and female gonads of ***O. nigricormis***.

## MATERIALS AND METHODS

For the present studies, ***Odontopus nigricornis*** were collected from the field area of Horticultural Experiments and Training center, Saharanpur. The fifth instar nymphs of the pest of approximately same size and age along with fresh

leaves and seeds of ***P. alata*** were collected manually by hand picking method from the field area in the pin-holed aerated polyether bags (30 x 20 cms). These were brought alive in the laboratory for their mass culture and restored in large wooden wire gauze cages untill they molted into the imagos. The newly emerged pairs of imagos were sorted out from this mass culture and kept separately for further rearing in the hurricane glass lamp chimneys as well as in wooden wire gauze cages till they molted into the images. The newly emerged pairs of images were sorted out from this mass culture and kept separately for further rearing in the hurricanes glass lamp chimneys as well as in wooden wire gauze cages.

**Preparation of Extracts of Test Plants**

500 gm leaves (newly emerged, immature, young and mature foliage) of each test plant ***Azadirachta indica*** (Neem) and ***Ocimum sanctum*** (Tulsi) were collected from the respective plant. Leaves were separately cleaned, shed dried and powdered by the electric grinder. The crude powders were dissolved in cold acetone, filtered carefully and finally stored in the refrigerator and used as test solutions for the experiments.

The desired concentration of each plant extract was obtained by dilution of the extract of the equal volumes in acetone to get the concentration of 2.0 percent of each extract.

**Application of Extracts of Test Plants on the Bug**

To assess the effect of plant extracts of ***A.indica*** and ***O. sanactum*** on reproductive organs of mature and freshly mated adults of either sex of ***O. nigricornis***; three sets each of twenty individuals of one-day old fifth instar nymph were employed for carrying out the experiments. A constant dosage Volume of 1µl of 20 percent concentration of extract of leaves of each test plant was topically applied ventrolaterally in the abdomen by Hamilton Repeater Syringe. After 6 hrs of treatment shock, the nymphs were kept in different batches in rearing chimneys observation were made every 24 hours of posttreatment untill imago emergence.

**Anatomical Study of Reproductive Organs of the Bugs**

The study of the morphology of the reproductive organs of Controls and test extracts treated male and female bugs, which were reared in chimneys in a laboratory was carried out by several dissections. These bugs were fixed in alcoholic Bouin's fluid for four to six hours, then washed three times in 70% alcohol and preserved in the same grade of alcohol. The preserved insects were mopped well in filter paper and after cutting their wings, these were fixed in insect wax trays. Dissections were done under the stereoscopic binocular microscope. The testes and ovaries of these bugs were cleared, taken out in 30% alcohol in cavity block. These were passed through the series of alcohol grades for dehydration. Some of the so dehydrated sets were also utilized for the microtomy and histology purpose.

**Histological Study of Reproductive Organs of the Bugs**

Some of the above-dehydrated sets of testes and ovaries of bugs were processed for microtomy blocks, using pure bee-wax, having melting point range between 55 to $60^0$ C. After routine processing, 8 to 10 µm thick sections were cut and stained in Heidenhain's Iron Haematoxylin-Eosin for the histological studies.

## RESULTS AND DISCUSSIONS

Effect of the extracts of leaves of ***Azadirachta indica*** and ***Ocimum sanctum*** on the development, growth, and maturation of gonads in the adults of male-female of ***Odontopus nigiricornis*** has been studied in the present investigations; Extracts of leaves of both test plants showed a significant inhibitory effect on the growth and maturation of gonads in adult of male and female of the bugs.

**Effect of Extracts on the Morphology of Reproductive Organs of Odontopus Nigricornis**

**Effect on the Morphology of Ovaries in Female O. Nigricornis**

The mature and mated adults of females of ***O. nigricornis*** which developed from female imago that have closed from fifth instar nymphs in controls showed the structure, growth, and maturation of ovaries similar to normal female individuals. In case of control-I (acetone treatment), the ovaries are elongated, turgid and compact in shape with average size (length x width) and weight being as13.21 x 2.30 mm. and 0.006 mg. respectively, whereas in case of control-II (without treatment), the average size and weight of ovaries were found as 13.26 x 2.31 mm, and 0.006 mg. respectively.

However, the mature and mated adults of females that developed from female imagos which have eclosed from fifth instar nymphs following treatment with 2 percent concentration of extract of leaves of test plant ***A. indica,*** the ovaries have been found to be much shrinked in shape, smaller, narrower, lighter, loose in appearance and on the average reduced to 12.36 x 1.81 mm. in size and 0.003 mg in weight.

In case of treatment with the extract of leaves of test plant ***O. sanctum*** having the concentration of 2 percent, the ovaries were found on the average to be of 12.70 x 2.02 mm. in size and 0.004 mg in weight.

The results of investigations depicted that there is no significant difference in size and weight of ovaries in either of the Controls.

The effect to the treatment of extracts of both test plants at 2 percent concentration showed a significant morphological suppressive effect on the shape, size, weight, and growth of ovaries of adults of female bugs, when compared to control—I and control-II. Moreover, the effect of an extract of leaves of ***A. indica*** on the morphology of ovaries of adults of female bugs was found to be more suppressive and inhibitory than the effect of ***O. sanctum*** as well as both of the Controls.

**Effect on the Morphology of Testes in Male of O. Nigricornis**

The development, growth, and maturation of testes in the mature and mated bugs of males of ***O. nigricornis*** which developed from imago that have emerged from fifth instar nymphs in Controls were found to be similar to normal male bugs. The average size (length and width) and weight of tests in the mature and mated of male bugs in control-I (Acetone treatment) were 3.08 x 1.82 mm and 0.003 mg respectively and in case of control-II (without treatment), it was 3.10 x 1.90 mm and 0.003 mg respectively.

In the mature and mated adults of male bugs that developed from male imagos which eclosed from fifth nymphs following treatment with extract of leaves of test plant ***A. indica*** having 2 percent concentration, the testes were found as more compact in appearance, shrinked in shape and reduced in dimensions on the average to 2.45 x 1.60 mm in size and

0.001 mg in weight, whereas in case of treatment with extract of leaves of ***O. sanctum*** having concentration of 2 percent, the testes were found on the average 2.72 x 1.71 mm in size and 0.002 mg in weight.

The result of the experiment showed that the extracts of both test plants of 2 percent concentration have a significant inhibitory morphological effect on the shape, size, weight, and growth of testes of male bugs when compared to control-I and II. Further, the effect of an extract of ***A. indica*** on the morphology of testes of male bugs was found to be more suppressive than that of ***O. sanctum*** as well as controls.

The effect of treatment of extracts of both test plants at 2 percent concentration showed the significant morphological suppressive effect on the shape, size, weight, and growth of ovaries of adults of female ***O. nigricornis*** when compared to Control-I and Control-II. Moreover, the effect of an extract of leaves of ***A. indica*** on the morphology of ovaries of adults of female bugs was found to be more suppressive and inhibitory than the effect of ***O. sanctum*** as well as both of the Controls.

**Effect of Extracts on the Histology of Gonads in O. Nigricornis**

In the present investigations, an attempt has been made to screen the disruptive effect (if any) of the test plant extracts of ***Azadirachta indica*** and ***Ocimum sanctum*** of 2.0 percent concentration on the gonads of mature-mated adults of both sexes of ***Odontopus nigricornis***. The results of the assessment are as under

**Effect on the Histology of Ovaries of Female O. Nigricornis**

The histology, growth, and development of ovaries and their ovarioles of mature and mated adults of females of ***O. nigricornis*** emerged from fifth instar nymphs in both of the Controls presented a generalized histological pattern similar to those found in other normal female bugs.

The ovaries of bugs in both controls (control-I in acetone and control-II untreated) appeared well developed, healthy, compact and elongated in shape and laden with yolk and oocytes. Each ovariole tapering anteriorly consists of a terminal filament, followed by germarium, the vitellarium, and a narrow neck or pedicel. The terminal filament at the apex of ovariole is a long fine filament. The syncytial epithelium of ovariole is best visible around germarium, which showed three histological areas of the zone. The apical part of germarium appeared to be filled mostly with small cells in various mitotic stages and some clusterings of cells. In the later zone of germarium, young oocytes are seen in the process of formation by aggregatation of trophocytes around enlarged nuclei. The vitellarium contains a row of oocytes in various stages of development. In vitellarium, the cells of follicular epithelium are full of granular cytoplasm, small scattered vacuoles, and yolky granules. Normal yolk deposition in the developing oocytes is seen. The basal area of vitellarium showed full packed yolk deposited oocytes, indicating the large size of oocytes.

The ovaries of mature-mated adults of females of ***O. nigricornis*** which have been obtained from imago after eclosion from fifth instar nymphs following treatment of extract of leaves of ***O.sanctum*** of 2.0 percent concentration showed several morpho-histological changes. The ovarioles contained fewer, smaller and deformed follicles. Some of the ovarian follicles exhibit disorganized epithelium at places, accompanied with shrinkage of their cytoplasm to some extent. In the vitellarium, the developing oocytes showed the moderate quantity of yolk deposition, lesser growth and decrease in size in contrast to the oocytes of female bugs in Controls. Few of the distorted oocytes

showed partial re-absorption, resulting in their failure of full development into the eggs.

However, the ovaries of mature-mated adults of female bugs obtained from female imago which has enclosed from fifth instar nymphs following treatment of extract of leaves of test plant of ***A. indica*** having 2.0 percent of concentration showed more pronounced histological effect. The ovaries were observed significantly reduced in shape, size, width, and weight by shrinkage. The terminal filaments of the ovaries appeared somewhat wavy and twisted. The anterior part of germarium contained lesser number of cells in mitotic stages and trophocytes.

The lumen of vitellarium contained number of much smaller and distorted ovarian follicles that too with shrinked cytoplasm and disorganized epithelium at places and poor yolk deposition is seen in the developing oocytes in the vitellarium region, resulting in a decrease in the size of developed oocytes.

The result of experiments showed, that in case of both Controls, no significant effect in histological structures in ovaries of female bugs has been observed. Treatments of extracts of both the test plants exhibited a significant inhibitory effect on the histology of ovarioles of bugs. Further, the disruptive effect of an extract of the test plant ***A.indica*** on the histology of ovaries is comparatively much severe than that of Controls and ***O. sanctum.***

Rajendran and Gopalan (1980) found that treatment of ***Dysdercus cingalatus*** with extract of ***Polycias quilfoylei*** adversely affected the size, weight, and development of ovaries, ovarioles, a number of ovarioles, and maturation of eggs. Likewise Verma (2004) tested the effect of leaves extract with 4 concentrations of ***Cassia fistula*** and ***Eucalyptus globulus*** on the gonadial maturation of ***Dysdercus koenigii*** and observed smaller ovaries with fewer degenerated follicles, decreased size of oocytes; disorganized follicle epithelium, decreased size of oocyte due to little yolk deposition and inhibition of Vitellogenesis accompanied with shrinkage of ooplasm. The findings also support the observations of Mohammad ***et.al***. (2010) on red palm weevil, ***Rhynchophorus ferrugenius*** with the treatment of neem extract (50,100 and 500 ppm). The found destruction in germarium, trophocytes, ovarian follicles, follicular epithelium and abnormal distribution of yolk and vacuolization of ooplasm. Further, Sharaley ***et.al***. (2011) tested alcoholic extracts of five medicinal plants including species of ***Euphorbia*** and ***Eucalyptus*** on first instar nymph of acrid bug, ***Heteracris littoralis***. They observed degenerated histological changes in the ovary of treated adult female bug like degeneration of follicular epithelium vacuolization to fill cavities of ovarioles damaged yolk droplets, shrinkage of ooplasm, and disruption of meiosis, reduced vitellogenesis, the partial growth of oocytes and clumping of few eggs.

**Effect on the Histology of Testes of Male O. Nigricornis**

In both of the controls (control-I acetone and control-II untreated) the testes of mature mated adults of the male of ***O. nigricornis*** showed histological structures similar to normal bugs and no apparent difference has been observed.

In both Controls, the process of spermatogenesis goes on as in the testis of other normal male bugs. The epithelium of testicular follicles appeared normal, intact, cytoplasm is clear and interstitial cells and spermatozoa are seen in plenty. Densely packed waves of spermatocytes in various stages of development, later on, their differentiation into spermatids and finally their transformation into spermatozoa are apparently visible in the testes. The testes in Controlled adults of male bugs contained compactly packed mobile spermatozoa.

The testes of a mature and mated adult of the male of ***O. nigricornis*** obtained from male imago which has eclosed from fifth instar nymphs following treatment with the extract of test plant of ***Azadirachta indica*** of 2.0 percent concentration showed several suppressive histological changes in structures. The testicular follicles showed disrupted epithelium at places, vacuolization in the cytoplasm of epithelial cells and decreased a number of interstitial cells. The germinal epithelium produced very few spermatogonia that too with faintly stained nuclei. Some of the nuclei of spermatogonia showed clumping of chromosomes and most of the spermatogonia do not develop beyond the stage of spermatocytes. Some of these spermatocytes, as well as the spermatozoa, have been observed as malformed and loosely arranged in the lumen of testes.

However, the testes of mature and mated adults of male bugs obtained from male imago which have emerged from fifth instar nymphs following treatment with the extracts of test plant of ***Ocimum sanctum*** of 2.0 percent concentration showed lesser and fewer suppressive changes in the morphohistological structure of testes in comparison to that of neem extract. The epithelium of testicular follicles appeared disorganized at fewer places resulting in lesser number of formation of spermatogonia. The cytoplasm of follicular epithelial cells contained fewer and finer vacuoles. Very few of the spermatogonia showed chromosomal clumping. All these changes resulted in a decreased number of formation of spermatozoa. The testes showed the feebly loosed organization of spermatozoa.

The results of present investigations indicated that the treatment of extracts of both test plants showed the significant suppressive effect on the histology of testes of male bugs. Further, the results also showed that the effect of an extract of ***A. indica*** on the histology of testes of male bugs was more pronounced and suppressive in comparison to the treatment with the extract of leaves of ***O. sanctum***.

Earlier, several entomologists reported deformities in the testes of various insect species using botanicals. Shimuzn (1988) reported the suppressive effect of Azadirachtin on the tests of cabbage bug, ***Mamestra brassicae***, incapable of forming spermatocytes and spermatogenesis. Verma (2004) mentioned vacuolisation in testicular sacs, decreased interstitial cells, clumping of spermatogonial chromosomes, disorganization and immobility of spermatocytes in the testes of ***Dysdercus koenigni*** with treatment of 4 concentrations of ***Cassio fistula*** and ***Eucalyptus globules*** Mohammad ***et.al.*** (2010) tested Azadirachtin extract (50, 100 and 500 ppm) against red palm weevil ***Rhynchophorus ferrugineus*** and observed tests with narrow, smaller and reduced follicular contents severe reduction in number of bundles of spermatogonia and spermatocytes. Likewise, Sharaley ***et.al***. (2011) reported in the acrid bug, ***Heteracris littoralis*** with an alcoholic treatment of extract of 5 medicinal plants; In treated testes they observed loosening in germ cells, vacuolization, reduced mitosis in the germinal zone and cytoplasmic disintegration in spermatocytes in the growth zone. The transformation zone also showed clumping of spermatids, abnormal spermatozoa, and picnotic cells.

Thus, the findings of present studies displayed a profound systemic pesticidal efficacy for the extracts of the leaves of ***A. indica*** and ***O. sanctum*** on the reproductive organs of ***O.nigricormis***, a pest of ***P.alata.*** Both the extracts of test plants were seen having an inhibitory effect on egg formation and suppressing the gamete-producing machinery as well as the reproductive potential of adult bugs. The test plants are available in abundance throughout India and thus, these can be easily utilized for management of this insect pest.

## ACKNOWLEDGEMENT

The authors feel obliged to the Principal and head of Zoology Department, M.S.College, Saharanpur 247001 as well as to the Director H.R.I and training center, Saharanpur for facilities of study site areas from where the collection of bugs was made.

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