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## Effects of Medicinal Plant Extract of Neem and Tulsi on Reproductive Organs and Reproduction in *Tenebrio Molitor* (Tenebrionidae)

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### Abstract

The *Tenebriomolitor*, is one of the most frequent insect pest of stored grains, and is a vector of many allergen factors. Thus, concerning public health and food safety, the control of this insect is appealing important. Dietary use of extract of neem and tulsi, which disrupt the development of reproductive organ, is incredibly new technique to manipulate this insect pest. In this study, the effect of dietary mixture of neem and tulsi was investigated on these insects. The control corporations acquired a normal food plan with no neem and tulsi mixture and each of the cure organizations obtained 10ppm, 30ppm, 50ppm, 100ppm, or 300 ppm of neem and tulsi combination in their diets, respectively. For every dose, 20larva have been used and the experiment used to be replicated three times. Each of the experimental repeats had a separate control group. The therapy period used to be 21 days, and then the insects had been fed with a regular food plan till they commenced to reach the pupal stage and finally emerged into an adult. At this stage, the dealt with adults have been kept with an untreated contrary sex till the formation of the first egg capsule. Use of neem and tulsi mixture was determined to be high quality in inducing abnormalities in growth such as retard growth 83.7% in males and 91.3% in female population. Furthermore, dietary use of this mixture brought about the sterility of the insects due to incomplete development of the internal reproductive organs. Various morphologic abnormalities like deformation and degeneration was located in the ovaries and accessory glands of sterile insects. Histological investigation of testis and ovaries in infertile insects indicated a degeneration of ovarian follicle cells, defective vital logenesis and reduce in the numbers of spermatocysts and spermatozoa. The exterior genitalia of the sterile insects in both sexes was not drastically altered in contrast with the controls.

**Keywords:** *Tenebriomolitor*, Neem & Tulsi Extract, Reproductive Organ, Sterility.

### Introduction

Mealworm as a massive pest for the grains storage, although it is used as a meals (Park et.al, 2014). Powdered larva product of yellow mealworm used as supplement over common ingredients as it comprise twice the higher content of protein, minerals and fats (siemianowska et.al.,2013). Yellow mealworm fed on agricultural waste product is very properly alternative to gain the diet from that agricultural waste for human consumption, however the accrued pesticide in the course of their life cycle reasons health dangers to the people (Houbraken et.al.,2016). Due to massive use of synthetic pesticide it precipitated insecticide resistance, environmental pollution and even it goals advisable bugs and no doubt its very detrimental for the people if consumed, so the interest closer to the herbal product is in growing demand now a days (Copping and Menn 2000; Isman 2006). Many pests damage the stored food merchandise which are saved in darkish and damp places. *Tenebriomolitor* commonly diagnosed as yellow meal worm. It is measured as a chief pest of saved meals grains. Polystyrene presently can't be decayed via dumping in landfills or recycled because of its complicated molecular structure. Annually, people throw away over 21 million tons of polystyrene. Previous research accomplished at Universities worldwide has located that *TenebrioMolitor* (mealworms)

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can potentially digest polystyrene safely through a intestine bacterium called *Exiguobacterium* sp. (Lomoriello et al., 2020). They choose to feed on food grains and food components nearing decaying stage. *Tenebriomolitor* can be honestly managed by fumigation with ethylene dichloride, carbon tetrachloride, methyl bromide or many different fumigants. But these tactics of fumigation are unsafe for nature due to the fact they may additionally harm human physique if the infected meals products are bump off via the human beings. Many pesticides are also acknowledged to purpose many physiological aberrations (Brown, 1963, Gangrade and Pant 1970), whether or not they are given in the shape of liquid spray or dust. Generally pesticides act a poison at the mobile level. Hence when stored meals grains are uncovered to positive insecticide as noted beforehand may also additionally end up dangerous to human beings deprived of killing the insect pests. DDT, BHC and Malathion which account for more than 50 percentage of total production are the most inexpensive and most famous with small farmers. Neem powder is water soluble and can be easily washed away and it is nice on unique pests and parasites such as ticks, mites, beetles (*tenebriomolitor*), bugs, cockroaches, head lice and many extra insects and pests (Schmahl et al., 2010). Neem stem twig broadly used as brushing (chewing) in rural region due to the fact immemorial time is proved to have antibacterial and anti-plague properties (Almas et al., 1995) insecticidal and pesticidal properties additionally pronounced in Tulsi leaf extract (Nanasombat S., Lohasuothawee P., 2005). Other method of manipulate like hormonal insecticide (Williams 1967) or Biological controls of different insect pests are being explored. The manages of Japanes beetle *papilla japonica* (Patterson et al 1992) is managed by means of their alkaloids produce through *Acromonium* spp. Alkaloids from N-lolii and N-perenne are in a position to alter the insect behavior (Azevedo J.L. 2000). Hassan and Shaw (2020) have described the effect of medicinal Plant Neem and Tulsi on the control of stored grain pest. The uses of above methods are harmful due to the fact it not only damage physiology of animals however additionally to the human beings. Biological pesticides having least lethal effect, fast degradation and reduced environmental negative properties make botanical pesticides as appropriate for natural farming (cosimi et al. 2008). The yellow mealworm is the largest of the insect species that attack the saved grain in India.

#### Materials and Methods

*Tenebriomolitor* was reared at room temperature ( $26 \pm 2^\circ$  C), and were fed by means of wheat grains. The larva of the insect was used to check out the effects of Neem and Tulsi extract combination blended with the wheat grain meals for every set of experiments. For every repeat of experiment, 120 larva were bought and allotted into six groups and each group has 20 larva. The manage group received ordinary food and remaining other five groups obtained 10ppm, 30ppm, 50ppm, 100ppm and 300ppm mixture of neem and tulsi extract in the

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diet, respectively. This compound was once dissolved in acetone to prepare the desired concentrations. The dietary therapy length was 21 days and then, all the mealworm had been fed a normal diet till they commenced to reach the pupal stage and transform into reproducing adult. The experiments were replicated three times and for each of the repeats a separate manage crew used to be utilized. In order to study the results of neem and tulsi on the growth of reproductive organ and reproduction, each dealt with insect was kept with an untreated contrary sex in a separate jar till the formation of the first egg capsule. Then, the fertility or infertility of the capsule used to be studied. To look into the results of neem and tulsi extract combination on the interior shape of the ovaries and testes of the insects. These organs were fixed in Bouin's solution (formalin 25%, picric acid 75% and acetic acid 5%) for 12 hours and then dehydrated. The organs were embedded in paraffin, sectioned and stained with hematoxylin-eosine and their histological abnormalities were studied.

#### Data Analysis

The statistical data were analyzed with descriptive facts the usage of the SPSS bundle version 13. The statistical methods are used totally based on the frequency of distribution, Chi square ( $\chi^2$ ) and nonlinear logistic regression.

#### Results and Discussion

##### Effect on Population Growth

The population of *Tenebriomolitor* shows abnormal growth such as retard growth and smaller in size. The sterile egg capsules were discoloured and wrinkly developed. With the growing concentrations of neem and tulsi aggregate the share of abnormalities were significantly ( $P \leq 0.01$ ) increased in each sexes. At a concentration of 300ppm, 83.7% of male and 91.3% of females were toward abnormal in condition. (Table 1)

##### Effect on Reproduction

The outcomes of neem and tulsi mixture on reproduction were investigated involving ordinary and abnormal *Tenebriomolitor*, whereas slowly sterile abnormal body growth appeared at 100ppm and 300ppm treatment (Table 2). The proportion of sterility was once significantly ( $P \leq 0.01$ ) multiplied in both sex (Table 2). According to the results, the incidence of female sterility at decrease doses of ( $\leq 30$ ppm) neem and tulsi extract combination indicates that female was affected greater severely than males.

##### Correlation between Sterility and Retarded Growth

The logistic regression analysis of the information available no vast variations between sterility and abnormal growth of each male and female insects ( $P \leq 0.05$ ). Thus, to learn about the relationship between abnormality and sterility, all the facts were accumulated. The outcomes indicated that the proportion of sterility in abnormal insects used to be drastically greater than normal ones. Thus, there is a significant ( $P \leq 0.01$ ) correlation between infertility and morphological abnormalities (Table 3).

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**Effect on External and Internal Reproductive Organs of Adult Female**

In comparison with the controls, the presence of a neem and tulsi extract combination had led to the incomplete improvement of ovaries and exceptional abnormalities such deteriorated ovary with partially developed eggs. In some cases, the external part of the oviduct was considerably wrinkled. The ovary was degenerated in some parts at 10ppm of food plan. Growth and improvement of the ovaries were certainly inhibited with 300ppm food regimen. This concentration caused the complete degeneration of the accessory glands. Application of neem and tulsi extract combination were also avoided the ordinary improvement ovarioles with induced abnormality and disruption of epithelial layer. This compound also caused the lysis of the nucleus of the ovarioles. In addition, the absorption of yolk in the eggs of the treated insects used to be incomplete due to the disruption of the formation of epithelium layer. Moreover, these vacuoles led to the degeneration of the nucleus of oocytes causing the sterility in adult females.

**Effect on External and Internal Reproductive Organs of Adult Males**

Neem and tulsi extract combination did not show morphological alterations on the testis and sperm ducts of the sterile male *Tenebriomolitor* in all treated groups. In some cases, abnormal and degenerated accessory glands were observed. The histological examination of testes of sterile *Tenebriomolitor* confirmed excessive degeneration & disruption of the testis walls as well as peritoneum membrane. Neem and tulsi extract mixture were disrupted the spermatogenesis and altered development of the sperms in adult males. In the present study, the dwarf *Tenebriomolitor* was sterile from each sex at 100 ppm (62%) and 300 ppm (65.5%) of neem and tulsi mixture. In addition, with the increasing doses of neem and tulsi extract mixture, the segment of retarded growth used to be significantly extended ( $P \leq 0.01$ ). As proven in Table 2, the low concentrations of neem and tulsi extract mixture i.e. 10ppm and 30ppm, were investigated the greater in all the reason the sterility of females compared with males. Application of neem and

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tulsi extract combination were confirmed a strong wonderful correlation between abnormal growth and the sterility of *Tenebriomolitor*. Therefore, the probability of sterility in abnormal insect is extensively ( $P \leq 0.01$ ) higher than the controls (Table 3). The morphological body growth and histological consequences of neem and tulsi combination on the reproductive organs of *Tenebriomolitor* was additionally investigated in the present study. Ovary, accessory glands and oviducts were degenerated, deteriorated and abnormally developed in adult insects. In some cases, the development of ovaries honestly inhibited. Neem and tulsi extract mixture caused some histological deformities on ovaries together with the degeneration of the testicular follicle epithelium, the incomplete deposition of yolk and the presence of empty cavities or autophagic vacuoles in the oocytes of ovarioles. In this study, neem and tulsi combination induced the formation of autophagic cavities in the oocytes inducing reabsorption of the yolk, digestion of proteins, and degeneration of the nucleus and consequently, infertility of the eggs. No huge morphological adjustments was determined on the testis and spermiduct of the sterile males, then again histological examinations of the testis of infertile insects indicated hypertrophy of the testis wall, the incomplete improvement of sperm, with a reduced number of spermatocyte and spermatozoa. The cytogenetic toxicity of the leaf extract of neem which was evaluated in murine germ. In which, substantial extended in the frequency of sperms with bizarre head morphology and the limit in simply sperm be counted were moreover observed (Khan & Awasthy, 2003). Herbal practise used as medicinal reason on account that from historical instances as mentioned in vedas was once an essential vital part of 'medicinal science in historic India' (Rastogi & Mehrotra, 2002). Recent observation about on Protein of yellow mealworm showed that special actions of pepsin and trypsin expressively decreased with age from the first harvesting time until 50 and 45 day after harvesting, respectively (Rodjaroen et al., 2020). No vast modifications had been found in the morphology of the exterior genitalia of both sexes, even at the greater treatment dose (300ppm).

**Table 1. Effect of Neem and Tulsi extract on the larva of *Tenebriomolitor***

Concentration (ppm)	Male Phenotype				Female Phenotype			
	Number	Normal growth	Abnormal growth	% Abnormality	Number	Normal growth	Abnormal growth	% Abnormality
0	36	36	0	0	24	24	0	0
10	34	33	1	2.9	26	22	4	15.3
30	35	27	8	22.8	25	17	8	32
50	32	18	14	43.7	28	12	16	57.1
100	34	10	24	70.5	26	4	22	84.6
300	37	4	31	83.7	23	2	21	91.3

The relationship between concentration and % abnormality is significant ( $P \leq 0.01$ ;  $\chi^2$  test).

Fig-1 Graph showing abnormalities in male and female *T. molitor*.

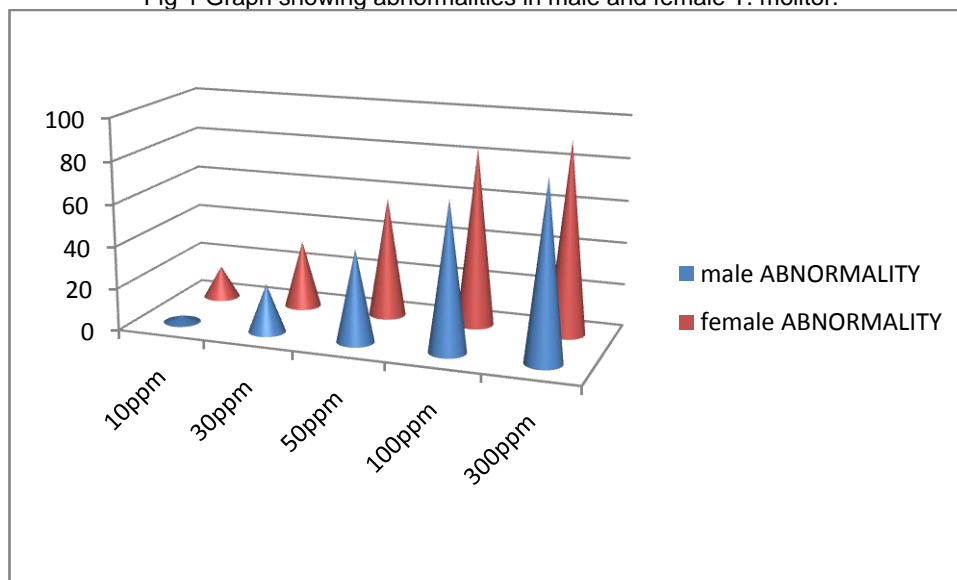


Table 2: Effect of Neem and Tulsi extract on reproduction of adult *Tenebriomolitor*

Concentration (Ppm)	Male				Female			
	Total Male	Normal Sterile	Abnormal Sterile	%Sterility	Total Female	Normal Sterile	Abnormal Sterile	%Sterility
0	36	0	0	0	24	0	0	0
10	34	1	1	5.8	26	1	2	11.5
30	35	6	4	28.5	25	4	6	40
50	32	7	10	53.1	28	8	10	64.3
100	34	10	17	79.4	26	9	14	88.5
300	37	12	22	91.9	23	2	21	91.3

The relationship between concentration and % sterility is significant, ( $P \leq 0.01$ ;  $\chi^2$  test).

Fig-2 : Graph showing sterility in Male and female *T.molitor*

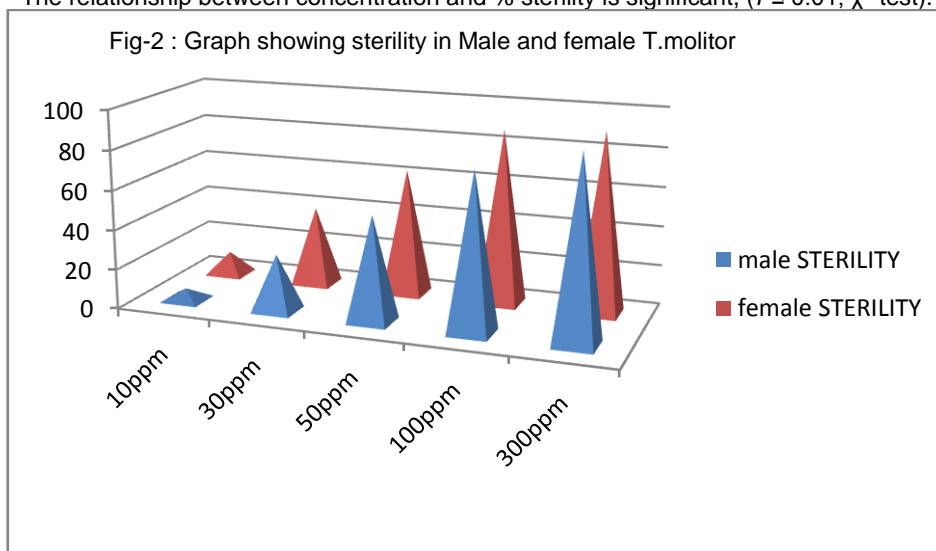


Table 3. The percentage of sterility in normal and abnormal phenotypes of *Tenebriomolitor*

Concentration (ppm)	Total normal (M+F)	Normal sterile	%Normality Sterile <sup>a</sup>	Total abnormal (M+F)	Abnormal sterile	%Abnormality Sterile <sup>a</sup>
10	57	2	3.5	5	3	60
30	50	10	20	20	10	50
50	40	15	37.5	34	20	58.9
100	29	19	65.5	50	31	62
300	24	19	79.2	55	36	65.5

Table shows the probability of sterility in abnormal insect is extensively ( $P \leq 0.01$ ) higher than the controls (Table 3).

**Aim of the Study**

1. To develop harmless control measures for the stored grain pests *Tenebrio molitor* by using extracts of Neem (*Azadirachta indica*) and Tulsi (*Ocimum sanctum*).
2. These extracts are being used to see the effects on Reproductive system and Reproduction of *Tenebrio molitor*.

**Conclusion**

According to the effects of this study and other reports, the application of neem and tulsi mixture on the *Tenebrio molitor* not only induces morphological growth deformities. However also causes the sterility of adults. This may also be due to the unfavourable outcomes of these compounds on reproductive organ development. Present investigation on larva & mature of *Tenebriomolitor* males and females using the extract of neem and tulsi had lowering effect in the growth of ovary and testis. This accordingly shows effect on the decline in ovarian protein concentration of larva and adult. This is in accord with the finding of Haque&Venkat Roman (1970) and Schmahal et.al (2010).The future lookup must additionally check out the effects on proteins content of larva as well as adult insect and also the effect of environmental parameters on them.

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