

Effects of Neem (*Azadirachta indic*) and Custard Apple (*Annona reticulata*) Diets on Sterility of House Rat (*Rattus rattus*)

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ABSTRACT

Three different plant products diets – i) neem (*Azadirachta indic* A. Juss) oil mixed diet (neem oil mixed @ 80 ml/kg of normal diet), ii) neem seed powder mixed diet (neem seed powder mixed @ 80 g/kg of normal diet) and iii) custard apple (*Annona reticulata* L.) seed powder mixed diet (custard apple seed powder mixed @ 80 g/kg of normal diet) were separately fed to mature rats (*Rattus rattus*) with single dose feeding of 80 g per pair in a day on 13th week-age during the experimenting years, 2012/013 and 2013/014. In control group only normal diet without neem and custard apple constituents were fed. Sterility test of rat was conducted up to 38 and 28 weeks-age in first and second year, respectively. The test rats were fed normal diet during whole experimenting periods except the one day when they were fed only the neem or custard apple mixed diet on the age of 13th week. Efficacy of the mixed diets on rat-sterility was determined based on pregnancy and parturition by the rats. The two years' results confirmed that all the tested three mixed diets – neem oil mixed diet, neem seed powder mixed diet, and custard apple seed powder mixed diet were effective to stop pregnancy and parturition in rats during whole experimenting periods up to 38 and 28 weeks-age with single dose feeding of 80 g per pair (40 gm/rat) in a day on 13th week-age of the rats; whereas the pregnancy and parturition were observed in the rats that were fed only the normal diet. It is expected, neem and custard apple mixed diets can be utilized in reducing the economically important rodent populations in rice-wheat cropping system in future.

Key words: House rat, rat sterility, neem oil, neem seed powder, custard apple seed powder

सारांश

मुसालाई नपुंसक तुल्याउन तीन थरिका वानस्पतिक आहारा (निमको तेल, निमको बिउको धुलो, सरिफाको बिउको धुलो) मिश्रत दानाहरु छुट्टाछुट्टै खाई मुसाको बाँभोपनमा पर्ने प्रभावकारीताको प्रयोग २०६९/१०७० र २०७०/०७१ मा गरिएको थियो। उक्त परीक्षणमा दानाहरु तीन बटा र एउटा वानस्पतिक पदार्थ बिनाको दाना तयार गरी १३ हप्ता पुगेका पाके उमेरका मुसालाई १३ हप्ता उमेर पुगेको दिन ८० ग्राम प्रति जोडी (१ भाले र १ पोथी) का दरले घरमुसालाई प्रयोगशालामा खाईएको थियो। अरु दिन परिक्षण अबधिभर मुसालाई वानस्पतिक पदार्थ मिश्रण नगरिएको दाना खाईएको थियो। परीक्षण कार्य मुसाको उमेर आ. व. २०६९/१०७० मा ३८ र २०७०/०७१ मा २८ हप्ता पुग्दा सम्म संचालन गरिएको थियो। मुसाको बाँभोपनको जाँच मुसामा गर्भ रहने तथा सन्तान उत्पादन गर्ने क्षमताको आधारमा गरिएको थियो। दुई वर्षको परिक्षणबाट प्राप्त नतिजाको आधारमा प्रयोग गरिएका तीन बटै (निमको तेल मिश्रत दाना (निमको तेल ८० मि.लि. प्रति किलोग्राम दाना), निमको बिउको धुलो मिश्रत दाना (निमको बिउको धुलो ८० ग्राम प्रति किलोग्राम दाना) र सरिफाको बिउको धुलो मिश्रत दाना (सरिफाको बिउको धुलो ८० ग्राम प्रति किलोग्राम दाना) ले ८० ग्राम प्रति जोडी तथा ४० ग्राम प्रति मुसाका दरले मुसालाई १३ हप्ता पुगेको दिन एकपटक मात्रै खाईएका परिक्षण अबधिभर मुसामा गर्भ र सन्तान उत्पादन रोक्न प्रभावकारी हुने पाईयो। जबकी वानस्पतिक पदार्थ मिश्रण नगरिएको दाना खाईएका मुसामा नियमित रुपमा गर्भ तथा सन्तान उत्पादन भएको पाईयो। यो प्रविधि भविष्यमा खास गरी धान पछि गहुँ लगाउने खेती प्रणालीमा मुसा नियन्त्रण गर्न उपयोगी हुन सक्ने सम्भावना देखिन्छ।

INTRODUCTION

More than 6000 species of rodents are reported worldwide among which 600 species belonging to genus *Rattus* are called rats (Fall 1977). Thirty species of rodents (rat and mice) are reported in Nepal out of them five are the economically most important common field and domestic rats and mice to damage the agricultural produce in fields and households (Joshi et al, 1991). They are the: house rat (*Rattus rattus*), house mouse (*Mus musculus*), brown rat (*Rattus norvegicus*), lesser bandicoot rat (*Bandicota bengalensis*) and large bandicoot rat (*Bandicota indica*) (Joshi et al 1991). Study on reproductive biology (Shrestha 2001) showed that successful mating of house rat was better in spring (90%) and autumn (90%) as compared to summer (80%) and winter (70%). In those studies, gestation period of house rat was 20-23 days; and male and female were sexually matured on 11th and 13th week after birth, respectively.

Enormous losses is caused by rodents in various ways by - damaging the standing crops and stored products; picking the sown seeds in fields; carrying the food materials in their burrows; spoiling the food grains with their excreta, urine and hair; extensive burrowing the crop fields; damaging the cloths and weaker storage structures; transmitting the rodent born diseases to human and pet animals etc (Joshi et al 1991, Ghosh and Durbey 2003, Lakshminarayanan et al 2015). In India, postharvest loss due to rodents was estimated to be 25-30%. In a 100 x 100 m² godowns, the loss due to rodents could be 4,200 kg estimating that 6 rats can consume the food equal to one man (Ghosh and Durbey 2003).

Rodents mainly the *Rattus rattus*, *Bandicota bengalensis*, and *Mus musculus* are agriculturally more important in Nepal causing the agricultural losses up to 15-25% every year (Shrestha 2001). Shrestha (2001) showed that the movement of house rat in rice and wheat fields in periphery of houses was up to 100 and 150 m, respectively. Yield losses caused by rodents in rice and wheat fields were estimated to be 575 kg/ha and 1132 kg/ha, respectively (Shrestha 2001). The total grain loss in storage by rat was estimated to be 2996 mt per year in Kathmandu valley (Shrestha 2001).

Several methods are available to control rats in fields and households (Joshi et al 1991, Upadhyay et al 1993, ED 1995/96, Mahajan et al 2015). However, in present experiments to minimize the rodent populations in fields and households, we approached method to cause sterility in rats by using botanical-mixed diets. For the purpose series of preliminary works were done in the past (ED 2008, Shrestha 2010, ED 2012). Here, in this paper we elucidate efficacy of some botanical-mixed diets on sterility of house rat.

MATERIALS AND METHODS

Preparation of Test Rats

House rats (*Rattus rattus*) were used as test rats that were collected from the farmers' households. They were kept in cages singly for three weeks in laboratory condition and were reared by feeding the normal diet and water at Entomology Research Division, Khumaltar, Lalitpur, Nepal. After the female rats were recognized not to be pregnant each of them were reared together with a mature male in separate cage for 10 days. The pregnancy of the female rats was checked by pressing the thumb in abdominal cavity. The pregnant rats were reared singly in separate cages until parturition. The newly born young rats were reared together with their mother up to 28 days. The young rats were then separated from their mother and were reared in pair (1 male and 1 female) or in groups until they get matured on 90 days (13 weeks) to be used for sterility test.

Preparation of Normal and Botanical Mixed Diets

Neem (*Azadirachta indica* A. Juss) seeds and Neem oil were collected in July while Custard apple (*Annona reticulata* L.) seeds were collected from August to September from local market. These seeds were separately shade dried and crushed into powder. Normal diet was prepared by mixing the various ingredients at the proportion of 400 g of chicken feed (No: 3), 200 g of whole wheat meal, 200 g of grinded gram, 100 g of broken pieces of groundnut, 60 g of skim milk, 20 g of sodium chloride and 20 ml of mustard oil. The normal diet was used for two purposes - one for maintenance & mass production of experimental rats, and another for control diet to be used as a treatment in sterility test of the rats. The two ingredients - groundnut pieces and mustard oil may not be required for preparing the maintenance and mass production diet.

The normal diet was cooked in boiling water and fed to rats daily for maintenance and mass production. Three different diets - i) neem seed powder mixed diet (neem seed powder mixed @ 80 g per kg of normal diet), ii) custard apple seed powder mixed diet (custard apple seed powder mixed @ 80 g per kg of normal diet), and iii) neem oil mixed diet (neem oil mixed @ 80 ml per kg of normal diet) were prepared for efficacy test on rat-sterility. The four diets (one control/normal and three botanical mixed diets) that were to be used as four treatments in sterility test were prepared in the form of pellets each of 10 g dry weight (11.5 g of wet weight) with the help of pellet making frames. The wet pellets of 11.5 g were oven dried at 60°C for 5 hours or until to dry.

Test of Botanical-Mixed Diets on Sterility of House Rat

Each treatment diet was fed to four pairs (as four replications) of matured rats in a day on 13th week-age with single dose feeding of 80 g (8 pellets each of 10 g) per pair in laboratory/room condition during the experimenting years, 2012/013 (2069/2070) and 2013/014 (2070/2071). Each pair (1 male and 1 female) was kept in four separate cages as four replications - Cage 1, Cage 2, Cage 3 and Cage 4 (Tables 1, 2, 3, and 4). The experiment was conducted starting from 13th week-age and completing on 38th and 28th week-age of the rats, in 1st and 2nd year, respectively. The test rats were fed in normal diet during whole experimenting periods except the one day, when they were fed only the botanical-mixed diet on their 13th week-age. Rat-sterility was determined based on pregnancy and parturition by the rats. Pregnancy was checked by pressing the thumb in their abdominal cavity weekly. Body weight was also observed weekly. Parturition was observed based on gestation period regularly. The diet was supplied to rats at 3-4 PM daily.

RESULTS

During the two years' experiments no pregnancy and parturition was observed in the rats that were fed the neem oil, neem seed and custard apple seed mixed diets. All the tested three mixed diets found effective to stop pregnancy and parturition in rats during whole experimenting periods up to 38 & 28 weeks-age of the rats with single dose feeding of 80 g per pair in a day on 13th week-age of the rats (Tables 1, 2, 3). Whereas, the pregnancy and parturition were observed in the rats that were fed only the normal diets (Table 4). The results of test diets on rat sterility were same in 1st and 2nd year, therefore representative data of only 1st year has been presented here. All the tested mixed diets were effective to stop birth in rats in both years.

DISCUSSION

In preliminary studies (Shrestha 2010, ED 2008) all the three botanical-mixed diets - neem oil @ 20ml/kg of normal diet, neem seed powder @ 100 g/kg of normal diet, and custard apple seed powder @ 100 g/kg of normal diet were effective to stop birth in house rat during whole experimenting periods up to 40 weeks-age when each diet were fed @ 80 g per pair daily for two months in 2004/2005 and three months in 2005/2006. Later in a study (ED 2012) the same botanical-mixed diets that were used in the preliminary works were effective to stop birth in house rat and field rat (*Bendicota bengalensis*) during whole testing period up to 22 weeks-age when each botanical mixed diet were fed @ 80 g per pair daily for two weeks. Present study revealed that all the tested three botanical-mixed diets found effective to cause sterility in house rat during whole testing periods up to 38 and 28 weeks-age with single dose feeding @ 80 g per pair for one day on 13th week-age of the rats.

Table 1. Effect of neem seed powder mixed diet on pregnancy and parturition of house rat

Age in weeks	Cage 1			Cage 2			Cage 3			Cage 4		
	Body wt (g)		Preg-nancy	Body wt (g)		Preg-nancy	Body wt (g)		Preg-nancy	Body wt (g)		Preg-nancy
	Male	Female		Male	Female		Male	Female		Male	Female	
13	100	85	no	90	100	no	140	90	no	130	120	no
14	105	90	no	100	110	no	140	90	no	130	130	no
15	105	100	no	100	115	no	140	90	no	140	130	no
16	110	100	no	105	115	no	150	100	no	130	125	no
17	105	100	no	110	120	no	155	110	no	130	130	no
18	130	120	no	150	130	no	160	130	no	145	150	no
21	150	120	no	150	150	no	170	150	no	150	150	no
24	170	150	no	160	160	no	180	160	no	160	160	no
25	170	160	no	165	160	no	180	165	no	165	160	no
27	180	165	no	175	165	no	190	170	no	180	165	no
30	185	170	no	200	195	no	200	175	no	200	190	no
32	180	165	no	210	190	no	200	185	no	190	180	no
35	190	170	no	220	200	no	220	200	no	210	195	no
37	190	180	no	220	200	no	210	200	no	210	210	no
38	210	190	no	225	200	no	210	200	no	210	210	no

Table 2. Effect of custard apple seed powder mixed diet on pregnancy and parturition of house rat

Age in weeks	Cage 1			Cage 2			Cage 3			Cage 4		
	BW (g)		Preg-nancy	BW (g)		Preg-Nancy	BW (g)		Preg-nancy	BW(g)		Preg-nancy
	Male	Female		Male	Female		Male	Female		Male	Female	
13	190	80	no	13	110	No	100	80	no	120	90	no
14	100	85	no	14	110	No	100	90	no	140	80	no
15	110	110	no	15	120	No	100	110	no	140	100	no
16	120	100	no	16	125	No	120	100	no	135	95	no
17	125	95	no	17	135	No	140	95	no	135	95	no
18	140	120	no	18	140	No	150	120	no	160	110	no
21	150	140	no	21	160	No	170	140	no	170	125	no
24	160	150	no	24	170	No	200	180	no	190	140	no
25	180	160	no	25	180	No	210	170	no	190	150	no
27	190	170	no	27	190	No	220	185	no	200	170	no
30	210	180	no	30	200	No	210	180	no	180	180	no
32	220	180	no	32	200	No	210	185	no	200	190	no
35	220	195	no	35	210	No	210	170	no	200	195	no
37	220	195	no	37	220	No	210	175	no	200	195	no
38	220	190	no	38	210	No	230	180	no	200	200	no

Table 3. Effect of neem oil mixed diet on pregnancy and parturition of house rat (2012/013)

Age in weeks	Cage 1			Cage 2			Cage 3			Cage 4		
	BW(g)		Preg-nancy	BW (g)		Preg-Nancy	BW(g)		Preg-nancy	BW (g)		Preg-nancy
	Male	Female		Male	Female		Male	Female		Male	Female	
13	110	100	no	100	100	No	100	90	no	100	100	no
14	130	135	no	130	110	No	120	110	no	120	110	no
15	145	145	no	140	135	No	155	125	no	135	115	no
16	150	150	no	155	150	No	160	120	no	140	120	no
17	165	150	no	160	150	No	165	135	no	155	135	no
18	165	150	no	165	150	No	170	145	no	160	140	no
21	170	155	no	165	150	No	180	150	no	160	150	no
24	175	155	no	170	155	No	185	155	no	165	145	no
25	175	155	no	170	155	No	190	155	no	170	150	no
27	185	165	no	185	170	No	200	165	no	185	165	no
30	180	170	no	200	170	No	210	175	no	195	170	no
32	200	200	no	230	170	No	210	175	no	230	185	no
35	210	200	no	230	190	No	230	200	no	240	185	no
37	210	190	no	235	195	No	230	200	no	240	195	no
38	210	200	no	240	200	No	240	200	no	250	200	no

Table 4. Effect of normal diet on pregnancy and parturition of house rat

Age in weeks	Cage 1			Cage 2			Cage 3			Cage 4		
	Body wt (g)		Preg- nancy	Body wt (g)		Preg- nancy	Body wt (g)		Preg- nancy	Body wt (g)		Preg- nancy
	Male	Female		Male	Female		Male	Female		Male	Female	
13	110	100	no	100	100	no	110	100	no	100	100	no
15	125	120	no	120	120	yes	125	120	yes	130	130	yes
17	155	145	yes	135	165	yes	155	135	yes	145	165	yes
18	160	160	yes	140	135	par	160	170	yes	155	120	par
19	160	185	yes	155	145	no	160	130	par	165	165	yes
23	165	135	par	160	150	yes	160	185	yes	170	130	par
25	165	170	yes	166	160	yes	175	140	par	190	180	yes
27	175	140	par	175	185	yes	185	170	yes	195	135	par
28	190	145	no	185	140	par	195	130	par	190	140	no
29	220	160	yes	195	150	yes	210	140	no	200	155	no
30	220	175	yes	200	160	yes	220	150	yes	210	165	yes
32	220	195	yes	210	185	yes	220	180	yes	220	180	yes
35	230	170	par	235	160	par	230	155	par	200	195	yes
37	245	175	yes	210	170	no	210	160	no	200	165	par
38	250	180	yes	225	175	yes	215	165	yes	210	175	yes

Rodent damage is very serious to agricultural produce both in fields and households (Upadhyay et al 2093, Lakshminarayanan et al 2015). Mainly the house rat and field rats are more destructive to standing crops/crop produce, stored grains and particularly to rice-wheat cropping system in Nepal. This study has achieved a possible alternative approach to reduce rodent populations and minimize the yield losses in crop fields and households particularly in rice-wheat cropping fields by causing sterility in them. This study confirmed that all the tested three botanical-mixed (7.5% of botanical powder or oil mixed) diets – neem oil mixed diet, neem seed powder mixed diet and custard apple seed powder mixed diet found effective to stop birth in house rat for whole testing periods up to 38 and 28 weeks-age by single dose feeding of 80 g per pair (40 g/rat) in a day on 13th week-age of the rats.

Several studies have shown effect of plant materials which have been successfully used to cause sterility in rats (Upadhyay et al 1993, Lakshminarayanan 2015, Mahajan et al 2015). In addition, this study has provided the clear protocols to prepare the diets for experimental and mass production of rats and preparing of test rats which will be applicable for the future studies. The present study has shown a preliminary experiment causing sterility in rats by single day feeding of the specific ration; however, more detailed physiological evidence based experiments should be performed in near future.

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