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Enhancement of poison bait acceptance through taste additives in short-tailed mole rat, *Nesokia indica* (Gray) infesting datepalm orchards in Nok-kundi and Mashkale in province Balochistan, Pakistan

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Abstract

This study was planned to enhance the poison bait acceptance in short-tailed mole rat, *Nesokia indica* (Gray) by using different food additives. Chemical control is the most effective method for rat control but only difficulty is that rodents shy rapidly the poison bait. To overcome the drawback of bait shyness and to save food from rodents, led to the development of highly palatable formulation using taste additives (bait enhancers). Ten taste additives namely dates, egg, milk powder, brown sugar, yeast, glycerin, animal fat, fish meal, sunflower oil and minced meat were incorporated 5% by weight individually in a bait base made of broken rice and wheat flour. Under no-choice test, rats showed more preference to date additive bait as compared to other additives. Under paired choice test (two feeds), baits with date, animal fat and milk powder were more preferred. Similar trends were recorded in multiple feed choice tests. Date additive bait, which showed the most preferred from choice tests significantly, enhanced the acceptability by 69.21% and 62.09% of bait containing brodifacoum and bromadialone. The study revealed that the addition of 5% date in poison bait can be useful in enhancing bait acceptance by rats, reducing colossal losses to date-palm.

Key words: Taste additives, palatability, brodifacoum, bromadialone, *Nesokiaindica*

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Introduction

The short tailed mole rat (*Nesokia indica*; Gray, 1832) is a Palearctic rodent species and is widely distributed in Pakistan, India, Iran, Iraq, Egypt, Syria, Northern Arabia, Chinese Turkestan and Southern Russian Turkestan (Walker, 1975). In Pakistan *N. indica* is extremely widespread throughout the irrigated area of Sindh, Punjab (Khan, 1982; Fulk, et al., 1981) and in most of the broader valleys of Baluchistan province (Roberts, 1997). According to Taber et al. (1967), *N. indica* was originally confined to the margins of rivers above flood level, however, with introduction of irrigation canals, the cultivated parts emerged as suitable habitat and now *N. indica* is the most abundant mammal pest in Pakistan followed by *Bandicota bengalensis* (Gray). Survey conducted by the team of Vertebrate Pest Control Institute (VPCI), Southern-Zone Agricultural Research Center (SARC), Pakistan Agricultural Research Council (PARC), Karachi identified *N. indica* the main causative agent of date palm trees infestation, grown in Gwalishtap, Wadiyan and Rajay, district Chaghai and Mashkale area of district Kharan, Baluchistan. The farmers reported that incidence of rat outbreak first occurred across the border in Iran area during 1998. It seems that this species is transported in this area accidently and has adapted according to ecological condition of desert ecosystem and rapidly increased due to favorable climatic conditions and availability of plenty of nutritional food in the form of date-palm trees, their stems and fruits. Diurnal fluctuations of temperature and humidity, sparse vegetation, scanty rainfall, loose sand and strong winds are some of the important factors, which animals of the desert have to contend with. Adaptations to desert conditions may be anatomical as well as behavioural (Prakash, 1964). Damage up to 22.10% to old trees and suckers worth estimated economic losses of 10.66 million rupees annually for Nok-Kundi and Mashkale areas. Present study is based on in-depth laboratory study to develop highly palatable bait formulation for the successful control of mole rat (*N. indica*), thus to save the agro-based economy of the area. The loss is considered heavy in the presence of limiting factors in the desert environment as the date palm trees not only provide marketable dates but also are helping in stabilizing the sand dunes. Keeping in view the severity of problem and its effects on the biodiversity of the area, it is proposed that both Pakistan and Iran agricultural ministries jointly workout to design strategy to control the pest rodent from the date palm orchards for the betterment of farming community and the date industry of both countries. Several methods are being used in the world to minimize rodent losses but poison baiting is considered the main technique to control the rodent pests. Any rodent control program cannot be successful if the poison bait is not well accepted by the rats. Different species of rats differ in their diet and preferences. A thorough knowledge of the preferred food and feeding behavior of the pest species may be helpful in planning control strategies (Adamczewska, et al., 1979). In the present study, efforts have been made to enhance the acceptability (palatability) of single dose anticoagulant rodenticides (Brodifacoum and

Bromadialone 0.005% each) by mixing enhancer (taste additives) in the bait base to develop highly palatable bait formulation for the successful control of rodents infesting date palm orchards at Nok-Kundi and Mashkale areas in Baluchistan Province of Pakistan.

Materials and methods

Collection of rats: Short tailed mole rat, *N. indica* was live captured through watering the burrow tunnels and live trapping in the date-palm orchards of Nok-Kundi, Baluchistan. Capture through live trapping was not successful in this area due to high velocity dusty wind, which buried the traps under the soil. However one rat was live captured in single catch trap. It was observed that the rats were extraordinary bait and trap shy. These rats were seen roaming in day time under the canopy of sucker of date palm trees, while *N. indica* occasionally come out of their burrow.

Acclimation of rats: The rats, after arrival in the laboratory, were sexed and individually caged for acclimation for three weeks before undertaking various tests. Pregnant, lactating and sub-adults were discarded. The rats were fed on a laboratory diet containing wheat, rice, maize and fish meal before and between various tests. Water was provided ad-libitum.

Experimental design: The experiments were consists of no choice tests; free choice tests (paired choice and multiple feed choice tests); and, poison bait acceptance tests of Brodifacoum and Bromadialone (0.005% each) with and without taste additive. Under no-choice and choice tests, 10 rats (5 male and 5 female) were caged singly to perform the tests. The weight of the rats was recorded before the start of each test. Each bait, weighing 50 g was offered daily in especially designed feeding cups. The left over bait and spillage were collected at 24 h intervals each day, by placing blotting paper underneath the cages, and were weighed to calculate mean daily consumption with an accuracy of 0.1 g. Each day fresh bait was offered and the left over was discarded due to urine and fecal contamination by the rats. The position of feeding cups was changed daily to avoid any place preference trend. Each test lasted for 7 consecutive days. The rats were re-used in the tests and re-arranged in the subsequent groups, so that their previous test food exposure is evenly distributed among new groups. To further reduce carry over effects, 7 days rest periods were maintained between various tests. With poison bait acceptability test, 10 rats were offered poisoned bait with and without additive for 3 consecutive days in order to evaluate their relative acceptance. Although second generation anticoagulants require only single dose feeding. In the case of 3 days feeding was tested to obtain more reliable data.

Bait Preparation: In order to conduct no-choice and choice tests, bait with additive was prepared containing wheat flour 47.5% and broken rice 47.5% and taste additive 5%. Milk powder, brown sugar, egg (fresh) yeast powder, glycerin, animal fat (bovine), fish meal (powder), sunflower oil, minced meat powder, and fresh date (grinded) were used individually as taste enhancers (taste

additives). The poison bait containing 0.005% brodifacoum (2.5% master mix) was prepared using a mixture of wheat flour, broken rice, taste additive and poison in a ratio of (47.4:47.4:5:0.2). For comparative evaluation, poison bait without additive containing brodifacoum (0.005%) was prepared with a mixture of wheat flour, broken rice and poison in a ratio of (49.9:49.9:0.2).The poison bait with 0.005% bromadialone (0.25% master mix) was prepared by mixing wheat flour, broken rice, taste additive and poison in a ratio of (46.5:46.5:5:2) and poison bait without additive in the ratio of (49.0:49.0:2). The above mentioned materials were blended in an electric mixing machine adding enough water until a stiff dough was formed. Small pallets were prepared using an electric mincer with a sieve of the 11mm diameter. Baits were fan dried and stored in plastic bags.

Data analysis: Mean food consumption data was analyzed by one way analysis of variance with test food as the main factor. Where significant effects were found, the individual mean comparisons were made using Least Significant Difference (LSD) test at p= 0.05. In paired choice test, t-test was used to analyze the mean consumption of the treated (additives) versus plain bait. The same test was used in a choice test of poison bait acceptability, where the poison bait was kept as reference and the poison bait with additive as the test food. The percentage preference values were calculated by dividing the test food by the total food consumption and then multiplying by 100

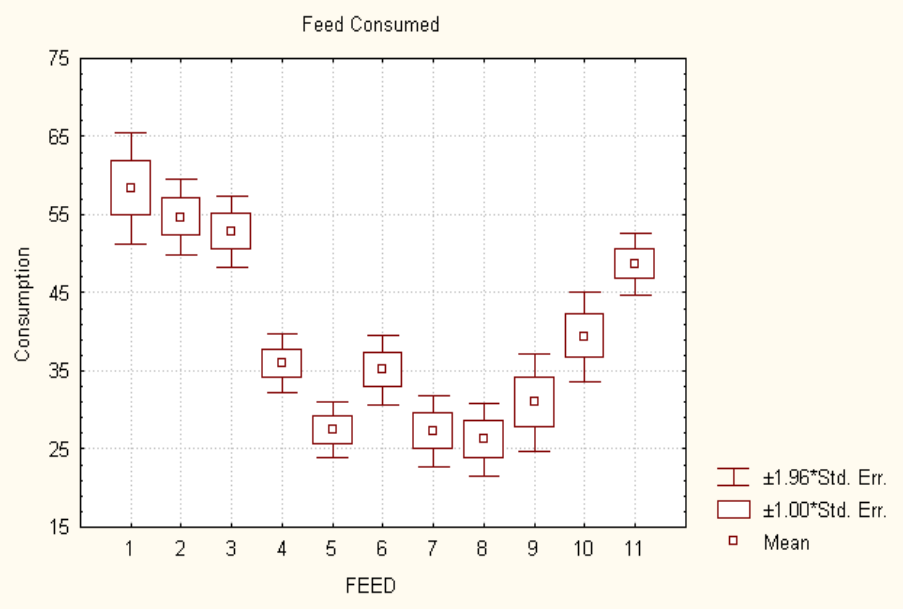

Figure 1: Feed consumption (g / kg); 1 = Date, 2 = Milk, 3 = Brown Sugar, 4 = Glycerin, 5 = Yeast, 6 = Egg, 7 = Animal Fat, 8 = Fish Meal, 9 = Sunflower Oil, 10 = Minced Meat, 11 = Plain.

Table 1. Mean daily consumption of various additive baits and plain bait under no choice test.

Bait additives	No. of animals (M /F)	Body weight (g) Mean ± SE	Average daily consumption (g / kg) ± SE
Date	10 (5/5)	299.86 ± 27.31	58.39 ± 3.61^{b}
Milk powder	10 (5/5)	293.83 ± 26.11	56.17 ± 2.69^{b}
Brown Sugar	10 (5/5)	308.38 ± 26.83	51.14 ± 1.84^{bck}
Egg	10 (5/5)	338.62 ± 28.92	35.92 ± 1.93^{dfij}
Yeast	10 (5/5)	385.42 ± 21.82	27.56 ± 1.64^{eghi}
Glycerin	10 (5/5)	351.75 ± 28.14	35.80 ± 2.42^{dfij}
Animal fat	10 (5/5)	361.01 ± 23.91	27.07 ± 2.44^{eghi}
Fish Meal	10 (5/5)	366.85 ± 27.72	26.21 ± 2.28^{eghi}
Sunflower oil	10 (5/5)	350.01 ± 30.52	30.95 ± 3.02^{efghi}
Minced meat	10 (5/5)	298.92 ± 28.17	39.44 ± 2.78^{dfj}
Plain	10 (5/5)	283.49 ± 34.17	48.67 ± 2.04^{ck}

Significant variation in consumption among various bait ($F = 22.09$; df effect=10; df error; t=98; $P < 0.01$) by ANOVA. Means followed by the same letter (s) are not significantly different at the 5% level by LSD

Results

No- Choice tests: No-choice tests were performed to ascertain if there is any potential for preference of additive baits compared with plain baits before undertaking regular tests. Date consumption is the highest though with larger variation. Only date, milk and brown sugar have greater mean consumption compare to plain bait (Figure 1). Analysis of variance (Table 1) Showed significant differences in consumption among various baits ($F = 22.09$, $P < 0.005$). Mean separation by LSD test revealed that baits containing date, milk powder and brown sugar additive were consumed significantly more than plain bait. Among additives, date bait consumption was maximum, while that of fish meal was minimum.

Choice tests: Paired choice tests were performed to examine mean daily consumption and percentage preference of 10 additive baits over plain bait as the alternative (Table 2). Baits with date, milk powder, and animal fat were significantly preferred ($P < 0.05$) over plain bait. Bait containing date additive was the most preferred (65.97%), while the bait with animal fat and milk powder were 2nd and 3rd in order of preference and the percentage of preference was 60.82% and 58.48%. Multiple feed choice tests (4 feeds) was performed to confirm or to re-establish the relative position of the 1st, 2nd and 3rd preferred bait additives emerged through period choice test. (Table 3).Analysis of variance revealed significant differences in bait intake ($F = 12.67$, $P < 0.001$). Significant difference and increase in consumption of date additive bait was recorded compared with animal fat, milk powder and plain baits. Date additive bait ranked first (45.85%) in relative preference to milk powder (24.08%), animal fat (19.96%) and reference (plain) bait (10.11%). The rats showed consistent preference for date over other additives. Similar studies conducted by Shafi et al. (1988) and Pervez et al.

(2000) revealed that texture and the particle size of the grain may play important role in enhancing the bait acceptance in *N. indica*. They recommended 2-3% addition of the egg shell in the bait base to make the texture of the bait more attractive and more palatable and to fulfill the requirement of calcium for the body need. In the agricultural fields, crop plants seem to be chief source of diet for mole rat *(N. indica*). In the sugarcane field of Sindh, as much as 89% of the rat's diet comprised of the cane alone (Smiet, et al., 1980). Similarly in the paddy fields, the rat intensively consumed the rice grain at harvest (Fulk, et al., 1980). In the orchards of Baluchistan about 93% of the diet of the rat was due to plants and the remaining 7% was due to insects (Mian, et al., 1987). The study showed that mole rat in the non-crop land of Baluchistan is largely herbivorous in diet (Ahmed, et al., 2007; Hussain, et al., 1995; Tousif, et al., 1985; Smiet, et al., 1980). The date fruit has high nutrient contents, which is energy rich in protein and carbohydrate etc. The mole rat (*N. indica*), being largely a subterranean species has to do a lot of digging to form long tunnels due to their fossorial habit to link with far apart other trees, which is an energy consuming act (Hussain, et al., 1995). So, it is assumed that the addition of the date fruit (5%) in the bait made it more palatable due to its high nutrient contents, sweetness and specific aroma of date fruit. The choice of food selection in rats is very complicated and may depend on the calorigenic contents (Hausmann, 1932), palatability (Young, 1946), behavioral components (Barnett, 1956), and daily energy requirements (Krishna Kumari, 1973). According to Krishna kumari (1968), less nourishing food are accepted if they are "Tasty" with flavor or soft texture. The selection is aided if the alternative food has distinct "taste". Evidently the physiological effect of a favorable food is associated with its particular taste and flavor (Barnett & Prakash, 1976). In the present study the preference for the date additive may be due to its palatability (Soft texture, sweet taste or specific flavor of date fruit.

Table 2: Mean daily consumption and percentage preference for various additive baits over plain bait as the alternative in paired choice tests.

Bait additives	No. of animals	Body weight(g) Mean ± SE	Average daily consumption (g / kg)		Percentage preference (Additive)
			Additive Mean ± SE	Plain Mean ± SE	
Date vs Plain**	10	283.19 ± 38.77	34.31 ± 4.38	17.70 ± 3.65	65.97
Milk powder vs plain*	10	313.76 ± 27.10	27.89 ± 3.16	19.00 ± 2.58	58.48
Brown sugar vs plain	10	362.24 ± 24.47	16.52 ± 4.98	15.40 ± 2.14	51.75
Egg vs plain	10	339.14 ± 28.88	20.25 ± 2.00	17.83 ± 1.15	53.18
Glycerin vs Plain	10	372.93 ± 23.46	15.35 ± 1.90	13.87 ± 2.73	52.53
Yeast vs plain	10	405.45 ± 18.13	12.79 ± 1.18	14.78 ± 1.83	46.39
Fish meal vs plain	10	371.40 ± 27.85	10.23 ± 2.76	24.16 ± 1.81	29.75
Animal fat vs plain	10	360.04 ± 23.95	16.84 ± 2.16	10.85 ± 1.28	60.82
Sunflower oil vs plain	10	356.22 ± 28.55	18.69 ± 2.75	21.38 ± 2.45	46.64
Minced meat vs plain	10	303.14 ± 29.29	20.43 ± 1.62	21.84 ± 2.60	48.33

*Significant at $P < 0.01$; $t = 2.7$; $df = 18$; **Significant at $P < 0.05$; $t = 3.05$; $df = 18$.

Table 3: Mean daily consumption and percentage preference of additive baits over plain bait as the alternative in 4 feed choice tests.

Feed items	No. of animals(*n*) (M/F)	Mean body weight of rats ± SE *(g)*	Mean daily consumption ± SE *(g/kg body wt.)* *, **	Percent preference
Date vs	10 (5/5)	330.88±30.08	14.95 ± 1.64[aa]	45.85
Milk powder			7.85 ± 1.68[b]	24.08
Animal Fat vs			6.51 ± 1.02[b]	19.96
Plain			3.30 ± 0.70[c]	10.11

*Significant difference in intake of different baits (F = 12.67; df effect = 03; df error = 46; P < 0.01); **Means followed by the same letter(s) are not significantly different at the 5% level of probability using LSD test.

Table 4: Relative poison bait acceptability of mole rat (*N. indica*) with and without additive (date) in a 3 day period choice test.

Poison	No. of animals (M/F)	Body weight (g) Mean ±SE	Mean poison bait consumption (g / kg body wt) ± SE		Percentage preference (Additive)
			With additive	Without additive	
Brodifacoum*	10 (5/5)	284.9 ± 36.89	43.11 ± 3.35	19.18 ± 2.93	69.21
Bromadialone**	10 (5/5)	313.76 ± 7.15	30.78 ± 1.60	18.79 ± 3.11	62.09

*****Significant at P < 0.01; t = 5.00; df = 18; ******Significant at P < 0.05; t = 2.00; df = 18.

Poison bait acceptability tests: The tests were carried out to ascertain the relative acceptance of poison bait with a highly preferred additive (date fruit) compared with the poison bait without additive (reference). Brodifacoum and bromadialone were used in the present study and have been showed to be highly effective against many rodent species from different ecological regions (Pervez, et al., 2000 and 1999; Maddaiah, et al., 1987; Mathur & Prakash, 1981; Brooks, et al., 1979). Result of a 3 days choice feeding study brodifacoum (P < 0.01) and bromadialone (P < 0.05) with additive (date fruit) compared with the poison bait without additive. Brodifacoum and bromadialone baits with additive were significantly preferred over poison bait without additive (69.21% and 62.09% (Table 4). Practically poison bait aversion trend in rodents can be overcome or minimized through making the more palatable by mixing the additives (Pervez & Khan, 2011; Shafi, et al., 1993). Similar results were achieved during laboratory study against *Rattus rattus* through changing the characteristic of bait or masking the smell and flavor of the toxins (Naheed & Khan, 1990). Earlier laboratory studies conducted on *B. bengalensis* and *N. indica* showed significant bait enhancement after additive of egg (Pervez, 2007; Shafi, et al., 1993). The present investigations clearly demonstrated that an addition of date fruit (5%) has the potential to mask the adverse effects in rats associated with unpalatable bait materials. Moreover, the highly palatable bait formulation has the advantage to address the control difficulties in field rodents due to constant availability of alternative food sources.

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