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Study of biological effects of Sodium Azide in M_1 generation and estimation of LD_{50} for growth and reproduction parameters

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Abstract
The purpose of this study was to carry out a comparative evaluation of the physicochemical properties of mucin powders extracted from three different sources.

Mucin powders was extracted from the African giant snails and the intestines of cow and pigs by the wet rendering process and differential precipitation with chilled acetone. The precipitates were air-dried and pulverized into powder. The powders were subjected to different organoleptic and physicochemical evaluations including solubility profiles, pH, moisture content and particle size. Their powder flow properties such as bulk and tapped densities, true density, angle of repose, flow rate, Hausner's ratio and Carr's compressibility index were also evaluated. The mucin powders showed comparable organoleptic properties, solubility profiles, melting points and pH. The powders were positive to tests for carbohydrates and proteins with traces of fixed oil. Microscopic examination of their particles reveals particle size and size distribution from 60 - 88 µm. There were slight variations in the bulk properties of the powders which exhibited good to fair flow ability with the following parameters; Hausner's ratio (1.11 - 1.30), Carr's index (9.99 - 18.44%), angles of repose (38.26 - 40.02°) and flow rate (2.68 - 3.25 g/sec). Their moisture content ranged from 10 - 16%. Results of the study has shown that the snail, bovine and porcine mucin powders are comparable in quality and thus may be considered equivalent when being used as an adjuvant in mucoadhesive drug delivery systems.

Keywords: Snail, bovine, porcine, physicochemical properties, mucin, mucoadhesives

Introduction
Among pulses, pea (*Pisum sativum* L.), also known as field pea and garden pea in English, and *Matar* in Hindi is one of the important *rabi* (winter) crops grown in the world and India. Pea (2n=2x=14) belongs to the family leguminoceae and genus Pisum. It is an annual herbaceous, self-pollinated crop. Field pea derives from the Middle East and was first cultivated roughly 10,000 years ago (Jing *et al.*, 2010) [10]. According to Blixt (1970) [6], the Mediterranean is the primary centre of diversity with secondary centres in Ethiopia. Fieldpea is one of the important pulse crop of India, grown in an area of 0.68 million hectares producing 0.62 MT of grain (Anonymous, 2011[3]. The average national productivity of field pea is 911 kg/ha. It is consumed as both green immature seeds as well as dry seeds. Availability of genetic variation is a pre-requisite for any crop improvement programme but pulses like pea generally lack genetic variability due to their autogamous nature. If enough variability does not pre-exist, then genetic variation can be created by several means; among which hybridization and induced mutation is one of the important methods. In pea, creation of variation through hybridization is tedious processes, due to highly self-pollinated, small, fragile flowers, that make it difficult to carry out the process of emasculation. Hence, the classical breeding methods have got limited application in pea and other pulses and as such, mutation breeding appears to play an important role in the improvement of this important pulse. So, as the primary objectives of mutation breeding is to enlarge the frequency and spectrum of mutations as an approach towards directed mutagenesis, the present investigation on study of biological effects of Sodium Azide in M_1 generation and estimation of LD_{50} for growth and reproduction parameters was carried out.

Materials and methods
The experimental field is situated at 24° 51'N latitudes 93° 56'E longitudes and at an altitude of 790 m above mean sea level. The climate of Imphal is sub-tropical with an average annual rainfall of about 1212 mm which is distributed mainly during the five monsoon months from

June to October. The mean annual maximum and minimum temperatures are 35ºC and 5ºC respectively. During the most part of the year humidity ranges from 70 to 80 percent with an average annual sunshine of about 2231 hours. The soil of the experimental site is clay in texture with acidic soil reaction. It is high in organic carbon content, medium in available nitrogen and phosphorus with high in available potassium. The pH of soil ranges from 5.60 to 6.80. Three genotypes of field pea namely Makhyatmubi, Makuchabi and Rachna were used in the present study. A chemical mutagen, sodium azide, a mono-functional alkylating agent was used in three concentration for induction of mutation in each variety. For Sodium Azide treatment, the selected seeds of each variety were divided into 4 lots which contain 375 seeds per lot in cloth bags. Out of 4 lots, one lot of seeds in cloth bag for all the genotypes were kept as control i.e., no treatment. The three remaining lots of each genotypes were used for Sodium Azide treatment. For chemical treatment, the seed lots were pre-soaked in distilled water for 6 hours before the treatment. One lot of pre-soaked seeds from each of the three genotypes was then subjected to 0.1%, 0.3% and 0.5% of Sodium Azide for 6 hours with intermittent shaking at room temperature 25±2 ºC Treated seeds of three field pea genotypes along with control were planted on raised beds with single seed per hill. The seeds were sown at 3-5 cm depth with 10 cm distance from plant to plant and 30 cm from row to row. Fertilizers were applied at the rate of 20: 40: 20 kg/ha NPK in the form of Urea (46% N), Single Super Phosphate (16% P_2O_5) and Muriate of Potash (60% K_2O) one day prior to planting in raised beds for better initial growth of the plant. After planting, irrigation was provided at proper interval for better establishment of the plants. Proper weeding and plant protection measures were taken up as and when required.

Result and discussion

Table 1: Effect of NaN_3 in M_1 generation of field pea genotypes.

Treatment	Germination %	Seedling height (cm)	Survival %
Makhyatmubi			
Control	79.95	12.33	79.21
0.1 % NaN_3	71.91	10.66	67.84
0.3 % NaN_3	70.22	9.66	49.93
0.5 % NaN_3	60.94	9.11	40.74
Makuchabi			
Control	80.22	12.16	71.51
0.1 % NaN_3	79.26	11.66	61.25
0.3 % NaN_3	74.67	9.16	59.90
0.5 % NaN_3	68.89	8.83	56.20
Rachna			
Control	93.89	12.83	69.22
0.1 % NaN_3	87.79	11.66	68.61
0.3 % NaN_3	78.26	10.96	66.20
0.5 % NaN_3	65.93	9.83	55.20

Seed germination (%)
Data obtained on effect of different concentrations of Sodium Azide on mean percent seed germination in control and mutagen treated pea cultivar is presented in Table. Analysis of variance for seed germination percent revealed that the variance due to genotype, dose and genotype x dose were significant. From the table it is evident that the percent seed germination in the cultivar subjected to treatment with different concentrations of Sodium Azide is definitely less than those of their respective controls. It clearly indicates that treatment with Sodium Azide have exerted an inhibitory effect on seed germination. The minimum germination percentage of 60.94 was recorded from 0.5% concentration of Sodium Azide in Makhyatmubi. Although mean germination was significantly reduced at all the concentrations as compared to the respective control for all the cultivars.These findings are in close agreement with the earlier reports of Govardhan and Lal (2013) [8] and Aney (2013) [2]. Similar inhibitory effect on seed germination by the mutagens have been reported earlier by Balai and Krishna (2009) [5] and Wani *et al.* (2011) [19] in mungbean, Sangle *et al.* (2011) [15] and Ariraman *et al.* (2014) [4] in pigeon pea, Karthika and Lakshmi (2007) [10] and Satpute and Fultambkar (2012) [16] in soybean.

Seedling height (cm)
The height of seedlings for three genotypes of field pea include in the study decreased with increasing concentration of Sodium Azide showing that seedling injury is positively correlated with concentration(Table 1). Similar decrease in seedling height with increased concentration of mutagen has been reported by Srivastava *et al.* (2008) [17] and, Govardhan and Lal (2013) [8] in pea, Perveen *et al.* (2012) in broad bean, Sinha and Lal (2007) in lenthil. Seedling height is widely used as an index in determining the biological effects of various mutagens in M_1 generation (Konzak *et al.*, 1965) [65]. Various explanations have been provided to explain the phenomenon of reduced seedling growth. Riley (1954) [14] suggested that it could be due to chromosomal abnormality with height reduction, reduction in auxin levels, inhibition of auxin synthesis, failure of assimilation mechanisms and chromosomal damage-cum-mitotic inhibition. According to Giri (2010) [7]the mutagen might inactivate the meristem, inhibit cell division, reduces number of cells contributing to seedling height and/or results in chromosomal damage leading to slow rate of meristematic action of shoot apex; resulting in reduced seedling height. Survival percentage (%) The analysis was shown percentage survival during the early growth stages of field pea showed an inverse relationship between concentration of Sodium Azide and survival percentage. Experimental results on effect of Sodium Azide on the three genotypes of field pea indicated that the percentage of survival of mutagen treated M_1 plants decreased with increase in the concentrations of the mutagens (Table 1). Highest number of plant survival was obtained from control (79.21 %) and a lowest number of survival percentage (40.74 %) from 0.5 NaN_3 treatment. Such a relationship between mutagen concentration and survival of plants has also been reported by Balai and Krishna (2009) [5] in mungbean; Sinha and Lal (2007) in lentil, and; Karthika and Lakshmi (2007) [10] in soyabean. The rapid infusion of chemical mutagen and their ability to produce chromosomal aberration and damage to genetic material is advocated by several workers as one of the causes for reduced survival percentage in M_1 generation after mutagen treatment. The genotypes responded differentially to various concentration of Sodium Azide. This indicates that effect of Sodium Azide on survival in field pea depends mostly on the genetic background of the plant receiving the mutagen.

LD_{50}
The LD_{50} of Sodium Azide for percentage reduction in seed germination for the three field pea genotypes Makhyatmubi, Makuchabi and Rachna and their graphical representation are presented in Table 2. From the table it is clear that the LD_{50}

value for germination percentage in all the three genotypes were exceeded the administered dose of Sodium Azide. The average LD_{50} value was calculated as 1.27% (Table 2). Rachna was seems to be highly sensitive to the Sodium Azide treatment as it recorded the lowest LD_{50} value i.e., 0.79% followed by Makuchabi and Makhyatmubi with 1.33% and 1.69% LD_{50} value respectively. The lowest LD_{50} dose, in the present study i.e., 0.79%, was estimated in Rachna variety (Table 2). This suggests that the variety Rachna is more sensitive than the other two genotypes to the Sodium Azide treatment. The difference in the LD_{50} dose among the genotypes of field pea used indicates that the sensitivity to Sodium Azide is dependent on genetic background of the population. The mean LD_{50} calculated taking average mortality induced in each dose was 1.27%. Similar findings were reported by Jain and Khandelwal (2009) while working on mungbean, Ali *et al*. (2012) [1] in rice and Prashant *et al*. (2016) in rapeseed-mustard. The dose much below 0.37% will definitely increase the availability of M_2 generation seeds but the mutation induced will not be satisfying and if the dose is more than 0.79% then enough population may not be available to grow M_2 generation. So, doses just above or equal to LD_{50} is suggested to use in further mutation program for inducing good mutations while insuring ample amount of individuals for screening those mutations.

Table 2: LD_{50} of three field pea genotypes**.**

Genotypes	LD_{50}
Makhyatmubi	1.69
Makuchabi	1.33
Rachna	0.79
Mean LD_{50}	1.27

Conclusion

From the present findings of the experiment it can be concluded that in M_1 generation characters like germination (%), seedling height (cm), survival percentage and plant height (cm) can be effectively used for estimating biological effect of mutagens. Biological effects on M_1 generation can be used as an advance indicator of the mutation taking place in the treated population. The mutagen Sodium Azide is highly effective in inducing genetic variability in yield contributing characters. Hence, induced mutagenesis can successfully be employed for induction of genetic variability among field pea genotypes which can either be used for selecting superior progenies among the population or can be employed in various hybridization programme.

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