

## STUDY ON IMPACT OF DIFFERENT CONCENTRATIONS OF SMOKELESS TOBACCO (GUTKHA) ON SEED GERMINATION, SEEDLING GROWTH, VIGOUR INDEX AND SEEDLING SURVIVAL OF *Vicia faba*

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### ABSTRACT

To observe the impact of smokeless tobacco, on different parameters of seeds of faba bean, (*Vicia faba*), pre-sterilized seeds were treated with four different concentrations of Gutkha, available in the market. After, 15 days of sowing the seeds, percentage germination was noted. At 0.25% concentration the percentage germination was 88.0%, at 0.50%, 80% at 0.75%, 56% and at 1.0%, 40% only. In control it was 96%, Minimum shoot length at 1.0% was taken which was 7.20 (cm), at 0.75% 8.58 cm, 0.50% 10.76 and at 0.25, 11.34 in the control it was 12.70. Similarly, root length at 1.0% was 5.25 cm, at 0.75% 5.70 cm, at 0.50% 6.15, and at 0.25% 7.38 cm in the control it was 8.56 cm. Seedling vigour index was 498.0 at 1.0%, 799.68 at 0.75%, 1352.80% at 0.50% and 1647.36 at 0.25% in the control it was 2040.96. Maximum seedling injury 20.0 and minimum percentage of survival was at 1.0%. At 0.75% the injury was 18.0 and survival 38%, 0.50% injury was 16.0 and survival 64, while at 0.25 the injury was 12.0 and survival 76.0. The maximum percentage of reduction in seed germination 58.34 was obtained among the seeds treated with 1.0% concentration. Similarly, maximum reduction in shoot length, 43.31%, in root length 38.67% in vigour index 75.60 and seedling survival 79.05 was obtained in 1.0% concentration. This was followed in 0.75% of concentration. Here minimum reductions in all the parameters considered here were obtained in 0.25% concentrations of Gutkha used here.

**KEY WORDS:** *Vicia faba*, Smokeless Tobacco, Gutkha, Seed Germination, Seedling Vigour, Seedling Injury

Now, it has been confirmed that use of tobacco is a main cause of oral cancer and preventable cause of premature death in India. Warnakulasuriya *et al*; (2005). IN recent years, a combination of areca nut and tobacco has been introduced in India known as Gutkha (Chewable tobacco). Gutkha is being consumed in most part of India as a mouth freshener (Balhi *et al*; 2009). Gupta (1999) commercially manufactured Gutkha consists of sundried, roasted, finely chopped tobacco, areca nut, slaked lime, and catechu. Different ingredients such as flavouring and sweeteners etc. are mixed with it. Ingredients of Gutkha contains different carcinogens. Among them tobacco specific N- Nitrosamines, 3- (methylnitrosoamino) propionitrilic (MNPN), and several other chemicals are present. Similarly, the Areca nut also contains, alkaloids, polyphenols, tannins, nitrosamine. In slaked lime the calcium hydroxide content of lime leads to alkaline conditions in the oral cavity with the presence of areca nut, which is a major factor responsible for the formation of reactive oxygen species. (ROS). It causes oxidative damage to different biomolecules in the cells. Similarly, the catechu, flavouring agents all are injurious to health. We get several references regarding the effects of Gutkha. Some of them are, Hoffman *et al*; (1994); Chaudhary (1999); Gupta and Warnakulasuriya (2002); Nair *et al*; (2004); Reddy and Gupta (2004); Chang *et al*; (2005); Stepnov *et al*; (2005); Mecht *et al*; (2007); Gupta *et al*; (2011); Karat *et al*; (2011); Bhisey (2012).

From the survey of literatures it was gathered that, pure form of different chemical mutagens has been used to observe their impact on seed germination seedling growth and other parameters in different crop plants. Some of them are being mentioned here, Abraham (1978); Sahu *et al*; (1981); Pandey *et al*; (1982); Parthasarthy (1983); Mishra and Singh (1986); Madhulkar (2002); Padavai and Dhanevel (2004); Toker (2004); Sharma *et al*; (2005); Singh and Kole (2005); Kavita Kumari *et al*; (2008); Bobhat and Dhema (2010). Meena (2011); Yazdani and Bagheri (2011); Bolbhat *et al*; (2012); Ariraman *et al*; (2014); Monica and Seetharaman (2015). These workers have used fungal extracts or chemical mutagens to observe their impact on seed germination, seedling growth, seedling vigour index and other parameters. Keeping these ideas in mind, the present was done to observe effect of smokeless tobacco on *Vicia faba* seeds for particular parameters such as inhibition of percentage seed germination, shoot and root length, seedling vigour, seedling injury and survival etc.

### MATERIALS AND METHODS

Seeds of *Vicia faba* were purchased from the registered seed traders at Muzaffarpur. Healthy seeds of uniform size were shorted from the seed stock. Seeds were surface sterilized with 0.5% Bavistin and then rinsed with distilled water several time to remove the chemical adhering on the surface. These seeds were dried

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with the help of pre-sterilized tissue paper. In the mean time, 1.0% solution of Gutkha powder was prepared the solution was warmed slightly for complete dissolution; the solution was filtered through double layered muslin cloth to separate the un-dissolved particles. This was the stock solution, and from it, 0.25, 0.5, 0.75% solutions were prepared by dilution. In four different 500 ml beakers, above solutions were transferred. In each, 50 seeds were soaked for 24 hours. Before this culture pots that was an earthen pots 12 inch height, top diameter 10 inch, base diameter 6 inch was taken. It was washed properly and sterilized with formaline 6%. Garden soil was taken. It was dried and powdered. All the coarse substances, debris of grasses were removed. Then they were also treated with formaldehyde. The soil was spread in sun on a well cleaned plastic for 8-10 hours to evaporate the formaldehyde. Next it was repeated again. Now the soil was mixed with vermicompost and placed in the earthen pot up to  $\frac{3}{4}$  parts. It was labeled properly and watered to amination moisture. Now 8-10 seeds were sown nearly 1.5 cm below the soil. All pots were kept

under laboratory conditions. Light was managed with the white fluorescent tube light. Pots were kept moist by spraying water. After 15<sup>th</sup> day the seed germination percentage was calculated for each treatment. Shoot and root lengths of the seedlings were measured after 3 weeks of sowing for this the seedlings were up rooted carefully. So that no part of the roots should remain in the soil. It was carefully washed and dried with the help of tissue paper. After measuring the length of the shoot and root the seedling vigour was determined by using the formula:-

#### Shoot length + Root length X % germination.

Seedling survival percentage was determined after 30<sup>th</sup> day of sowing the seeds in the pot. 50 seeds were used for each treatment. Each experiment was done in triplicate. The mean of the data of each parameter was placed in table-1, while the percentage of reduction by different concentrations of Gutkha in each parameter than that of the control was placed in table-2. The data were used for results and discussion. Distilled water treated seeds were used as control.

**Table 1: Showing four different concentrations of Smokeless tobacco (Gutkha) on different parameters of seeds of *Vicia faba*.**

Treatment (%)	No. of Seed sowing	Seedling shoot length (cm)	Seedling root length (cm)	Seedling Vigour Index	Seedling injury	Seedling survival %	Seed germination %
0.25	50	11.34	7.38	1647.36	12.0	76.0	88.30
0.50	50	10.76	6.15	1352.80	16.0	64.0	80.0
0.75	50	8.58	5.70	799.68	8.0	38.0	56.0
1.0	50	7.20	5.25	498.0	20.0	20.0	40.0
Control	50	12.70	8.56	2040.96	00	95.45	96.0

**Table 2: Showing % reduction in different parameters of seed after treatment with different concentrations of Gutkha in comparison to control.**

Treatment (%)	% of inhibition of seed germination	% reduction in shoot length	% reduction in root length	% reduction in vigour index	% reduction in survival
0.25	8.3	10.71	13.79	19.25	20.38
0.50	16.67	15.28	28.15	37.72	32.95
0.75	61.67	32.45	33.41	68.82	60.19
1.0	58.34	43.31	38.67	75.60	79.05
Control	00	00	00	00	00

## RESULTS

From the table, it was noted that the percentage of germination of seeds in control was 96%. However, the percentage of seed germination was reduced to 40%

when the seeds were treated with 1.0% solution of smokeless tobacco, the Gutkha. It was 56% in 0.75% and 80% in 0.50% concentration of the solution. Similarly, in control the shoot length was 12.70 cm, which was only

7.20 cm in the seedling where seeds were treated with 1.0% concentration. In 0.75% the shoot length of seedling was 8.58 cm and in 0.50, 10.76 cm. The minimum reduction in shoot length was in 0.25% that was 11.34 cm. In the control the root length was 8.56, which was only 5.25 cm in 1.0%, 5.70 cm in 0.75%, 6.15 in 0.50% and 7.38 in 0.25% of solution with which seeds were treated. The seedling vigour was the maximum, 2040.96 in control. While among the seedlings raised from the treated seeds it was 498.0 in 1.0% solution, 799.68 in 0.75%, 1352.80 in 0.50% and 164.36 in 0.25% concentration of the solution. Seedling injury was zero in the control, however, among treated, seedling it was 20 at 1.0%, 18 at 0.75%, 16.0 at 0.50%, and 12.0 at 0.25%. In the control the survival of seedling was 95.45% while among the treated it was 20 at 1.0%, 38.0 at 0.75%, 64.0 at 0.50% and 76.0 at 0.25% respectively.

From the above data, the percentage of reduction in seed germination, the shoot and root length, seedling index vigour, and seedling survival were calculated among the treated seeds with that of the control one. The mean of the data so obtained, was presented in table-2. From the table, it was noted that percentage of seed germination was only 58.34% when seeds were treated with 1.0% of the solution followed by 61.67 in 0.75%, Here minimum reduction only 8.3% was observed at 0.25% concentration of the solution. Similarly, maximum reduction in shoot length 43.31% was noted in 1.0% concentration of the solution, followed by 32.45% in 0.75% and only 10.71% in 0.25% concentration of the solution. From the table-2, it was noted that maximum reduction in seedling vigour was 79.05% in 1.0% concentration, followed by 60.19% in 0.75%, 32.95% in 0.50% and only 20.38% in 0.25% concentration.

## DISCUSSION

As mentioned above, Gutkha is a mixture of tobacco, Areca catechu, Areca, slaked lime, different flavourings and sweeteners. Most of them contain carcinogenic chemicals. When seeds were soaked with the solution of Gutkha, there might have been certain physiological disturbances, as is reported by Gaul (1970). Djordjevic (1994) reported tobacco specific N-Nitrosamines and Areca derived N-Nitrosamines both are carcinogenic. Yazdani and Bhagheri (2011) reported allelopathic effect of tobacco, on germination and early growth of Soybean, (*Glycine mase* L.). Bholbhat and Bhoge (2012) observed effect of mutagen on seed germination, plant survival and quantitative characters of horse gram (*Macrotyloma unifloram* UDRX). Ariraman

*et al*; (2014) observed mutagenic effect of certain chemical mutagens on seed germination, seedling growth and seedling survival of pigeon pea (*Cajanus cajan* L.). Similarly Monica and Seetharaman (1915) observed the impact of physical and chemical mutagens on seed germination and seedling growth on garden bean. All these workers concluded that in comparison to control, there was decrease in all parameters along with the increasing concentration of the mutagens. Therefore, present findings corroborate with the findings of the above workers, because here also, there was proportional decrease in all the parameters along with the increasing concentrations of the chemicals.

## CONCLUSION

It may be concluded that all the concentrations of Gutkha, called smokeless tobacco, had inhibitory effect on seed germination, seedling growth, seedling vigour index, survival percentage as compared with that of the control. If this small concentration had such impact on living cell physiology, what extend it can damage in case of human cells. So there should ban on its production, distribution and marketing.

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