Research Article

Effect of Chemical Mutagen on Seed Germination, Morphological and Essential Oil Content in *Ocimum basilicum* L.

Neha Kumari[1], Maneesha Singh[2],*

[1]Department of Botany, School of Basic and Applied Sciences, Shri Guru Ram Rai University, Patel Nagar, Dehradun, Uttarakhand, INDIA.
[2]Department of Botany, School of Basic and Applied Sciences, Shri Guru Ram Rai University, Dehradun, Uttarakhand, INDIA.

Submission Date: 22-08-2023; Revision Date: 14-09-2023; Accepted Date: 18-10-2023.

ABSTRACT

Sweet Basil (*Ocimum basilicum* L.) variability might be improved with hybrid combinations but combination breeding is a time-consuming process for genetic improvement of this useful plant due to smaller floral size and autogamy. Therefore, induced mutation is an important complementary breeding method for this plant to produce heritable changes both in the case of qualitative and quantitative traits. The present study has been carried out to induce variations in *Ocimum basilicum* L. using Ethyl Methane Sulphonate (EMS), a chemical mutagen with different doses to determine the rate of seed germination, survival rate, morphological characters, and essential oil content in expectation of finding desirable mutants. Results revealed that there were significant effects of EMS doses on seed germination, rate of plant survival at maturity, pollen viability, morphological characters and oil content. With the increase in EMS concentration, the rate of seed germination and pollen viability was significantly decreased. The present studies showed positive as well as negative effects of mutagenesis *i.e.* mutants with bushy appearance, increased height, number of primary branches and broad leaves, dwarf with small leaves, short internodes with few nodes, and reduction of seed size and ultimately low yield has been observed in *O. basilicum* L. Thus, these findings suggest that mutation breeding is an important method for improving crops and creating new genetic resources with desirable traits under diverse climatic conditions.

Keywords: *Ocimum basilicum* L., Ethyl Methane Sulphonate (EMS), Seed germination, Pollen viability, Essential oil.

Correspondence:
Dr. Maneesha Singh
Professor, Department of Botany, School of Basic and Applied Sciences, Shri Guru Ram Rai University, Patel Nagar, Dehradun-248001, Uttarakhand, INDIA.

Email: singhmaneesha2@gmail.com

INTRODUCTION

Ocimum basilicum L. commonly known as sweet basil belongs to the family Lamiaceae, and is an important annual culinary herb grown for both fresh and dry consumption. It is a source of essential oil for manufacturing perfumes, food flavours, and aromatherapy products.[1] This plant is cultivated in several countries, such as India, France, Morocco and Italy for commercial purposes.[2,3] The essential oils of *Ocimum basilicum* L. are rich in linalool, methyl chavicol, eugenol, 1, 8-cineone, methyl eugenol and anthocyanin. Due to the presence of several important phytoconstituents, the basil plant possesses many biological potentials such as anti-microbial, anti-viral, antioxidative, anti-cancer properties, etc.[4] Besides this, the plant is used for traditional and modern therapeutic values like cancer, convulsion, diarrhoea, epilepsy, gout, hiccup, toothaches, and whooping cough. It is important to know about genetic variability, adaptability, and evolution of the plant species for the success of crop improvement programs. Recently, several studies showed that basil variability might be improved with hybrid combinations but combination breeding is time-consuming process for genetic improvement of this useful plant due to smaller floral size and autogamy. Therefore, Induced mutation is an important complementary breeding method to produce

SCAN QR CODE TO VIEW ONLINE

www.ajbls.com

DOI: 10.5530/ajbls.2023.12.61

heritable changes both in the case of qualitative and quantitative traits.[5] Studies have been conducted on using physical as well as chemical mutagens to improve qualitative and quantitative characteristics in crops like cereals, pulses, vegetables, and medicinal and aromatic plants.[6-12] Our endeavour through the present studies was to generate and enhance the variability in basil plants using different concentrations of Ethyl Methane Sulphonate, a chemical mutagen. The resulting variants were analyzed in terms of seed germination, pollen viability, morphological and oil content alterations for creating high essential oil-yielding lines.

MATERIALS AND METHODS

Experimental Site and climatic condition

The present study was carried out from November 2019-May 2022 at the Research Laboratory in the Department of Botany, School of Basic and Applied Sciences and at the agriculture field of School of Agricultural Sciences, Shri Guru Ram Rai University, Patel Nagar, Dehradun, and Uttarakhand. Dehradun district is located between the latitude 29°55' and 38°31' N and longitude 77°35' and 78°20' with an elevation of 650 m above sea level. The climate of Dehradun is generally sub-tropical, although it varies from tropical to severely cold, depending upon the season, and the altitude of the area. During the summer, the temperature varies from 27-40°C, and during winter it is between 2-24°C. Constant and heavy rainfall occurs during the rainy season.

Materials

Seeds of *Ocimum basilicum* L. (CIM- Saumya variety) have been collected from the Central Institute of Medicinal and Aromatic Plants (CIMAP, CSIR) Lucknow, Uttar Pradesh.[13]

Treatment

Seeds were pre-soaked in distilled water for 3 hr and then treated with EMS at concentrations of 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9% and 1.%. Each concentration of EMS was prepared in 100 mL of distilled water with Dimethyl sulphoxide (DMSO) and 100 seeds (M0) were treated for 1, 2, 3, and 4 hr separately to increase the probability of generating mutants. LD_{50} of EMS treatment was calculated to induce mutation on a large scale in *Ocimum basilicum* L.

Preparation of field and planting of treated seeds

Land was prepared with proper ploughing followed by application of farm yard manure and treated seeds along with control was sown individually under suitable agro-climatic conditions to raise the M1 generation. 100 treated seeds from each concentration and treatment time combination were planted individually in raised beds of 1 m×4 m size in a field of 10-15 cm height with irrigation channels. The seeds are then covered with a thin layer of fine soil. This treated population was designated as the M1 population. 100 untreated seeds of the CIM-Saumya variety of *Ocimum basilicum* L. were planted in separate blocks for comparison of survival mutants in the field.

Seed germination and Pollen fertility

The number of seed germination in the treatments along with control has been recorded to calculate the rate of seed germination. Growth parameters such as seedling height, and rate of survival have been observed at one-month-grown plants.

Pollen fertility (%) has been determined by using the pollen grains from freshly dehisced anthers of M1 plants per treatment with iodine solution and five slides per treatment were observed under the microscope. The pollen grains which were stained properly were counted as fertile and others who have not taken any stain or, were not in proper shape were considered sterile.

Morphological Characterization

Different morphological characters viz., plant height, number of primary branches, stem girth, leaf length, leaf breadth, length of Inflorescence, canopy and seed weight along with control have been observed in the M1 generation.

Extraction of oil

The essential oil was extracted from 100 g of fresh leaves of all selected mutant plants by hydro-distillation method using a Clevenger apparatus. The extracted essential oil was stored at 4°C in dark airtight bottles for oil profiling.[14]

Statistical analysis

Mean ($\bar{X}$), Standard Error (SE), and Standard Deviation (SD), were done using R 3.1.0 and IBM SPSS statistics 20 to assess the intra- and inter-population (mutagen) variations in different quantitative traits.

RESULTS

In the present study, findings on seed germination, pollen viability, survival percentage morphological characters and oil yield have been discussed under the following heads:

Seed germination, percentage of plant survival at maturity, and pollen viability

The effect of different concentrations of Ethyl methane sulphonate on seed germination, seedling height, plant

survival and pollen fertility (%) has been studied in *Ocimum basilicum* variety CIM-Saumya. In the case of control plants, 95% seed germination was recorded whereas in the case of treatment, the rate of seed germination ranged from 56% to 82%. Maximum seed germination was reported in 0.1% EMS treatment (82%) and minimum in 1.0% treatment i.e. 56% (Table 1). The present study revealed that the average number of seed germination had reduced with increasing concentration of mutagens. Reversed inhibition has been observed in percent inhibition. Minimum inhibition was recorded at 0.1% and maximum at 1.0% (Table 1). In the case of 1% EMS treatment, seedlings showed distinct morphological variation at two or, four leaf stages. Seedlings showed curling of leaves, unequal length of leaves, and albino mutants were recorded at 1% EMS dose (Figure 1).

Seedlings' height ranged from 8.33 cm to 13.66 cm in all the treatments whereas 10.66 cm seedling height was reported in control plants at one-month maturity. Maximum seedling height was reported in 1.0% (13.66 cm) and minimum in (0.1%) (8.3 3 cm) treatments (Table 1). In control, 98% plant survival at one month of seedling maturity was recorded whereas it was ranged from 58% to 83% in treatment conditions. In 0.2% treatment maximum plant survival at maturity and minimum in 1.0%. Maximum maturity at one month seedling stage was reported in 0.1% and minimum in 1.0% EMS treatment (Table 1). LD_{50} of EMS treatment was reported at 1.2%. Therefore, for conducting the experiments lower concentration than 1.2% was used to induce mutation on a large scale in *Ocimum basilicum* L. and above 1.2% concentration was considered as a lethal dose.

The percentage of pollen fertility recorded ranged from 68 to 88% whereas a maximum in 0.1% and a minimum at 1.0%. Reversed inhibition has been recorded in percent inhibition. In the control plant, 92% pollen fertility (%) has been observed. Increasing doses of concentrations decrease pollen fertility with a maximum of 0.1% and a minimum of 1.0%.

Morphological characters and Essential oil contents

Screening of mutants was based on morphological variations in the plant height, length of third node,

Table 1: Effects of mutagens on seed germination, seedling height, plant survival at maturity (%) and pollen fertility.

Treatments dose of Mutagens	Seed Germination		Seedling Height		Plant Survival at Maturity		Pollen fertility	
	Actual Percentage	Percentage Inhibition	Actual (cm)±SE	Percentage Inhibition	Actual Percentage	Percentage Inhibition	Actual Percentage	Percentage Inhibition
Control	95	-	10.66±1.20	-	98	-	92	-
0.1%EMS+DMSO	82	13.68	8.33±1.22	21.85	83	15.30	88	4.347
0.2%EMS+DMSO	79	16.84	8.66±0.33	19.04	81	17.34	85	7.60
0.3%EMS+DMSO	76	20.00	10.33±0.33	3.09	79	19.38	84	8.69
0.4%EMS+DMSO	73	23.15	10.66±0.88	0	75	23.46	82	10.86
0.5%EMS+DMSO	71	25.26	11.33±0.33	-6.28	72	26.53	80	13.04
0.6%EMS+DMSO	68	28.42	11.66±0.33	-9.38	68	30.61	77	16.30
0.7%EMS+DMSO	65	31.57	12.33±0.66	-15.66	65	33.67	75	18.47
0.8%EMS+DMSO	63	33.68	12.66±0.88	-18.76	63	35.71	73	20.65
0.9%EMS+DMSO	60	36.84	13.33±0.33	-25.04	60	38.77	70	23.91
1.0%EMS+DMSO	56	41.05	13.66±0.66	-28.14	58	40.81	68	26.08

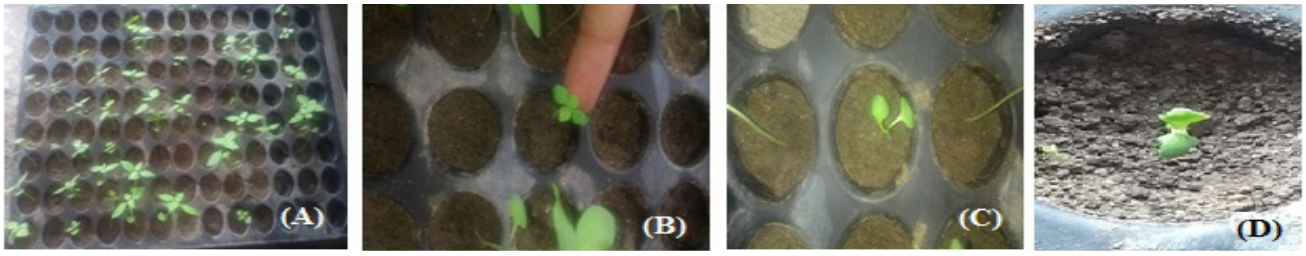

Figure 1: Germination of seedling showing morphological variation (A-mutant population; B- seedling with four leaves; C-seedling with two leaves; D-seedling curling leaf of 1% EMS dose).

stem girth (thickness), number of primary branches, leaf length, leaf breadth, length of inflorescence and canopy (Table 2). The plant height ranged from 74 cm to 125 cm. Maximum height has been observed in 1.0% and minimum in 0.1% of treatments. Stem girth varied from 3.2 to 6.5 cm at different EMS doses. A number of primary branches ranged from 6-18 as compared to the control. A minimum number of primary branches was recorded at 1.0% and a maximum in 0.8% of treatments. The maximum canopy recorded in 0.4% of EMS dose was 80-109 cm.

The leaf morphological variations were recorded in leaf length and breadth. Leaf length varied from 3.3-4.9 cm and leaf breadth ranged from 1.6-2.2 cm. Leaf length increased from 3.3 cm to 4.9 cm as compare to control (3.9 cm). Minimum leaf length was observed in 0.9% and maximum leaf length in 0.3% treatments. Leaf breadth increased as dose of EMS increases as 1.6 cm to 3.1 cm and 1.5 cm in control and minimum in 0.1% and maximum in 0.3% treatments.

EMS treatment affects the length of verticillaster inflorescence which was ranged from 10-28 cm. The variation in petal colour pigmentation has been observed in flowers of treated plants. Inflorescence length increases as dose concentrations increase, from 10 cm to 28 cm with a minimum at 0.5% and maximum at 0.2%. These findings revealed that there was a directly proportional relationship in inflorescence length with an increase in the dose concentration of EMS. In the high doses, increased seed size has been recorded. Seed

Table 2: Details of Morphological and oil related traits in EMS+DMSO treated M1 Population of *Ocimum basilicum* L.

Treatments dose of Mutagens	Mutants	Plant height (cm)	Stem girth (cm)	Number of primary branches	Leaf length (cm)	Leaf breadth (cm)	Inflorescences length (cm)	Canopy (cm)	Seed weight (gm)	Oil content (%)
Control	OB	95	4.9	21	3.9	1.5	20	29	18.30	0.6
0.1%	OB1a	101	3.2	08	4.6	1.9	30	18	11.42	1.0
EMS+	OB1b	110	4.7	12	4.6	3.2	39	82	16.65	0.8
DMSO	OB1c	74	6.5	18	4.0	1.5	13	50	14.25	0.6
	OB1d	105	3.0	06	3.5	1.6	13	96	22.17	0.7
0.2%	OB2a	105	4.0	13	4.2	1.6	28	78	27.77	0.9
EMS+	OB2b	105	5.8	12	4.5	2.1	20	89	10.22	1.6
DMSO	OB2c	98	4.0	08	4.4	2.2	23	63	18.56	0.6
0.3%	OB3a	115	4.2	07	4.9	2.2	17	86	14.15	0.4
EMS+	OB3b	107	4.7	14	4.0	3.1	22	88	35.68	0.9
DMSO	OB3c	110	5.0	10	4.4	1.7	18	98	16.88	0.5
0.4%	OB4a	124	6.0	12	3.6	1.7	16	109	20.36	1.2
EMS+ DMSO	OB4b	96	3.5	09	4.0	1.9	23	64	19.58	0.5
0.5%	OB5a	87	4.3	08	3.5	1.6	18	57	7.85	0.6
EMS+ DMSO	OB5b	101	3.5	09	3.9	2.0	10	69	10.69	0.4
0.6% EMS+ DMSO	OB6a	120	4.3	12	3.9	2.0	19	83	16.25	0.6
0.7%	OB7a	108	4.2	14	3.6	1.7	1.8	66	18.96	0.5
EMS+ DMSO	OB7b	110	5.0	10	3.8	1.9	20	96	11.66	0.7
0.8%	OB8a	97	5.0	18	4.4	1.9	20	98	30.70	1.4
EMS+ DMSO	OB8b	124	5.0	14	3.8	1.9	23	95	16.52	0.8
0.9%	OB9a	90	5.2	17	3.6	1.6	2.3	94	23.33	0.8
EMS+ DMSO	OB9b	117	6.0	14	3.3	1.9	1.5	17	16.77	0.6
1.0% EMS+ DMSO	OB10a	125	5.2	14	3.6	17	23	80	15.63	0.7

Figure 2: Variations in plant height compared to control showing high yielding, busy, erect and dwarf (Mutant: OB3a, OB5a, OB8b, OB4a, Control: OB).

Figure 3: Variations in leaf characters compared to control (OB) showing increased in leaf length (OB8a) and curling of leaves (OB 10a).

weight ranged from 10.69 gm to 35.68 gm whereas in the control plant, seed weight was 13.30 gm. Minimum seed weight was observed in 0.5% and maximum in 0.3% of treatments. The yield of seed increased recorded as an increase in the dose of concentration as 35.68 gm to seed 10.22 gm. Essential oil yield has been increased as compared with the control and varied from 0.4-1.6% (Table 2). Oil content increased along with control 0.6% and was recorded as varied from 0.5% to 1.6% with a minimum at 0.7% and a maximum at 0.2% dose treatments (Table 2). Thus, the present studies showed positive as well as negative effects of mutagenesis *i.e.* mutants with bushy appearance, increased height, number of primary branches and broad leaves (OB3a, OB4a) and a dwarf with small leaves, short internodes with few nodes, and reduction of seed size and ultimately low yield (OB5a, OB 8b) has been observed (Figures 2 and 3).

DISCUSSION

Genetic variability is the primary source for crop improvement programs. There are many ways to improve and produce better genotypes to achieve high production for food and health safety. The economic development of any country in the world is sustainable growth and the performance of agriculture and other sectors. This can be achieved by creating new varieties that are more productive and resistant to the adverse effects of climate change.[5] Among the many breeding methods, crop improvement through genetic modification and inducing variability has played an important role in improving and developing desirable and outstanding varieties of different crops. Mutation breeding is an efficient tool to induce variability and to isolate desirable agro-economic traits in a short duration compared with conventional breeding methods by using physical and chemical mutagenic agents. This method can be used in both self- and cross-pollinated crops.[15,16] Induced mutants and their role in recombinant breeding have facilitated the development and release of more than 458 mutant and mutant derivative varieties in India covering cereals, legumes, oilseeds, ornamental plants, medicinal and aromatic plants and other multi-agro-ecological crops. Mutant varieties are widely accepted and cultivated to increase production and economic welfare across the country.[5,8-11]

In the present studies, the effect of different concentrations of Ethyl methane sulphonate along with DMSO has been studied in *Ocimum basilicum* variety CIM-Saumya on both qualitative and quantitative characters like initial growth parameters, seed germination, pollen viability, plant height, stem girth (thickness), number of primary branches, leaf length, leaf breadth, length of inflorescence, floral petal pigmentation, canopy and essential oil content. These findings revealed that there was an inversely proportional relationship between the concentration of EMS and quantitative morphological parameters. Therefore, for conducting the experiments lower concentration than 1.2% was recommended to induce variations in commercial scale in *Ocimum basilicum* L. Comparative studies on control and treated populations revealed that variations have been observed at different doses of treatments due to mutagenesis. Similar studies have been observed in the case of leaf characters in lentil plants.[17]

Ample variations in the length of verticillaster inflorescence, petal colour pigmentation, seed yield,

seed size and essential oil content have been observed in comparison with control plants. Inflorescence length, seed size and total oil content have been increased as EMS concentrations increase. These findings showed that there was a positive relationship in inflorescence length, seed size and oil content with the dose of EMS. The present findings suggested that there were positive as well as negative effects of mutagenesis i.e. mutants with bushy appearance, increased height, number of primary branches and broad leaves (OB3a, OB4a) and dwarf with small leaves, short internodes with few nodes, and reduction of seed size and ultimately low yield (OB5a, OB 8b) has been observed. Several studies on medicinal and aromatic plants for developing high-yielding varieties have been carried out by Scientists all over the world. Some important varieties are CIMAP Sammohak of German Chamomile, Sujatha of Opium poppy, Niharika of psyllium etc.[18] Thus, variability in morphological and yield-related traits can be created by reshuffling the genetic background of a plant for developing high yielding varieties with wide adaptability with minimum requirements on cultivation for livelihood of farmers.

CONCLUSION

The present studies on mutagenesis showed that the action of chemical mutagen EMS with DMSO is an important method for improving crops and creating new genetic resources which can be adapted to diverse environmental conditions with higher yield in *Ocimum basilicum* L. can be utilized as a new high yielding variety for economic development of farmers.

ACKNOWLEDGEMENT

Authors are grateful and express sincerely thanks to Prof. (Dr.) Arun Kumar, Dean, School of Basic and Applied Sciences, Shri Guru Ram Rai University, Patel Nagar Campus, Dehradun for his kind support and suggestions.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR'S CONTRIBUTION

There is equal contribution of both the authors.

ABBREVIATIONS

EMS: Ethyl methane sulphonate**, DMSO:** Dimethyl sulphoside.

REFERENCES

1. Simon JE, Morales MR, Phippen WB, Vieira RF, Hao Z. Basil: A source or aroma compounds and a popular culinary and ornamental herb. In: Janick J, editor. Perspectives on new crops and new uses. Alexandria, VA: ASHS Press; 1999. p. 499-505.
2. da Costa AS, de Fatima Arrigoni-Blank M, Aparecida Azevedo Pereira da Silva M, Alves MF, Darlisson de Alexandria S, Alves PB, *et al*. The impact of hybridization on the volatile and sensorial profile of *Ocimum basilicum* L. Sci World J. 2014;8.
3. Malekpoor F, Pirbalouti AG, Salimi A. Effect of foliar application of chitosan on morphological and physiological characteristics of basil under reduced irrigation. Res Crops. 2016;17(2):354-9. doi: 10.5958/2348-7542.2016.00060.7.
4. Singh M, Kumari N. Botanical description, phytoconstituents, cultivation practices and future prospective of *Ocimum basilicum* L. JETIR. 2023;10(2):489-504.
5. Pérez-Escamilla R. Food security and the 2015-2030 sustainable development goals: from human to planetary health: perspectives and opinions. Curr Dev Nutr. 2017;1(7):e000513. doi: 10.3945/cdn.117.000513, PMID 29955711.
6. Sebastian SA, Fader GM, Ulrich JF, Forney DR, Chaleff RS. Semi dominant soybean mutation for resistance to sulfonylurea herbicides. Crop Sci. 1989;29(6):1403-8. doi: 10.2135/cropsci1989.0011183X002900060014x.
7. Thurling N, Depittayanan V. EMS induction of early flowering mutants in spring rape (*Brassica napus*). Plant Breed. 1992;108(3):177-84. doi: 10.1111/j.1439-0523.1992.tb00118.x.
8. Maan SS, Williams ND. An EMS induced dominant allele for male-sterility transferred to euplasmic wheat 1. Crop Sci. 1984;24(5):851-2. doi: 10.2135/cropsci1984.0011183X002400050005x.
9. Yudhvir S. Mutagenic effect of N-nitro-N methyl urea and ethyl methane sulphonate on the incidence of fruit rot in tomato. New Agric. 1995;6:89-94.
10. Saba N, Mirza B. Ethyl methane sulfonate induced genetic variability in *Lycopersicon esculentum*. Int J Agric Biol. 2002;4(1):89-92.
11. Sengupta S, Datta AK. Induced narrow leaf mutant of sesame (*Sesamum indicum* L.). –Indian. J Genet. 2005;65(1):59-60.
12. Enkhbileg E, Fenyvesi A, Bíró B, Fári MG, Kurucz E. Mutation induction in sweet basil (*Ocimum basilicum* L.) by fast neutron irradiation. Int J Hortic Sci. 2019;25(1-2):30-8. doi: 10.31421/IJHS/25/1-2./2912.
13. Lal RK. High yielding eugenol rich oil producing variety of *Ocimum sanctum* CIM- Ayu. J. Med. Arom. Plant Sci. 2003;25:746-7.
14. Singh A, Kumar A, Agrawal SP, Siddharth BR. Cultivation, Oil Extraction and Chemical Composition of Sweet basil (*Ocimum basilicum*) in Kannauj Region. Int J Innov Res Sci Eng Technol. 2017;6(11):1.
15. Yadav AK, Singh S, Dhyani D, Ahuja PS. A review on the improvement of *Stevia rebaudiana* Bertoni. Can J Plant Sci. 2011;91(1):1-27. doi: 10.4141/cjps10086.
16. Agrawal L, Kumar M. Improvement in ornamental, medicinal, and aromatic plants through induced mutation. J Appl Biol Biotechnol. 2021;9:162-9.
17. Dixit P, Dubey DK. Induced mutation in Lentil (*Lens culinaris* Medik.). Lens. Newsletter. 1983;10:7-10.
18. Lal RK, Chanotiya CS, Gupta P. Induced Mutation breeding for qualitative and quantitative Traits and varietal development in medicinal and aromatic crops at CSIR-CIMAP, Lucknow, India: past and Recent Accomplishment. Int J Radiat Biol. 2020;96(12):1513-27. doi: 10.1080/09553002.2020.1834161, PMID 33026919.

Cite this article: Kumari N, Singh M. Effect of Chemical Mutagen on Seed Germination, Morphological and Essential Oil Content in Ocimum basilicum L. Asian J Biol Life Sci. 2023;12(3):460-5.