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RESEARCH ARTICLE :

Effect of ethyl methane sulphonate (EMS) on sprouting and survival characteristics of garlic (*Allium sativum* L.)

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SUMMARY : The experiment was carried out to study the effect of ethyl methane sulphonate (EMS) on sprouting percentage, survival percentage and lethal dose (LD_{50}) of garlic (*Allium sativum* L.) in M_1 generation. There were three garlic genotypes IG-2010-3-2, IG-2009-11-1 and Agrifound White used for the experiment. Garlic cloves were treated with five different concentrations of ethyl methane sulphonate (EMS) *viz.* 0.1%, 0.4%, 0.8%, 1.2% and 1.6%. A field experiment was conducted during the year 2014-15 in Rabi season under All India Network Research Project on Onion and Garlic at Horticulture Instruction cum Research Farm of Department of Vegetable Science, Indira Gandhi Krishi Vishwavidyalaya, Raipur (C.G.). The result indicated that values of all parameters were decreased by increasing concentration of ethyl methane sulphonate (EMS). The LD_{50} values estimated based on the 50% reduction of germination and survival percentage result showed that effect of EMS concentration varies in different genotype.

KEY WORDS :
Garlic, Mutation, Ethyl methane sulphonate, Sprouting percentage, Survival percentage, LD_{50}

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BACKGROUND AND OBJECTIVES

Garlic (*Allium sativum* L.) is a bulbous herb used as a food item, spice and medicine in different parts of the world. Its medicinal use is based on traditional experience passed from generation to generation. Researchers from various disciplines are now directing their efforts towards discovering the effects of garlic on human health. Interest in garlic among researchers, particularly those in medical profession, has stemmed from the search for a drug that has a broad-spectrum therapeutic effect with minimal toxicity. Garlic extract has antimicrobial activity against many genera of bacteria, fungi and viruses. The role of garlic in preventing cardiovascular disease has been acclaimed by several authors. Chemical constituents of garlic have been investigated for treatment of hyperlipidemia, hypertension, platelet aggregation and blood fibrinolytic activity. For pest control garlic has strong insecticidal, nematicidal, rodenticidal and molluscicidal activity. Adverse effects of oral ingestion and topical exposure of garlic include body odor, allergic reactions, acceleration in the effects of anticoagulants and reduction in the efficacy of anti-AIDS drug Saquinavir.

Mutation is a sudden heritable change in organism generally the structural change in gene. It is produced by change in the base sequence of genes and it can be induced either spontaneously or artificially both in seed and vegetative propagated crops. Induced mutations have recently become the subject of biotechnology and molecular investigation leading to description of the structure and function of related genes. Induced mutations are highly effective in enhancing natural genetic resources and have been used in developing improved cultivars of cereals, fruits and other crops (Lee *et al.*, 2002). These mutations provide beneficial variation for practical plant breeding purpose. During the fast seven decades, more than 2252 mutant varieties of different crops have been officially released in world (Maluszynski *et al.*, 2000). A great majority of mutant varieties (64%) were developed by the use of gamma rays (Ahloowalia *et al.*, 2004). Hence, mutation-breeding programme has proved to be a successful tool in bringing amelioration in self-pollinated crops.

Chemical mutagenesis is a simple approach to create mutation in plants for their improvement of potential agronomic traits. Mutation methodology has been used to produce many cultivars with improved economic value and to study the genetics and plant developmental phenomena (Aruna and Adamu, 2010). Mutagens may cause genetic changes in an organism, break the linkages and produce many new promising traits for the improvement of crop plants. Among the chemical mutagens, EMS is reported to be the most effective and powerful mutagen (Shah *et al.*, 2008; Minocha and Arnason,1962; Hajra, 1979).

The frequency and type of produced mutations depends on the plant species or varieties, the dosage of mutagen, the situation of plant before, after and during of the induction. So, it is very important to have knowledge about the plant response or seedling behaviour to obtain successful variation through mutation. The LD_{50} use by the researcher to determine the lethal dose of mutagens (Warghat *et al.*, 2011 a; Talebi *et al.*, 2012 a; Anbarasan *et al.*, 2013 a). In each mutation breeding program initially LD_{50} is determined, which is used as an optimal concentration for induction. By ignoring this step, mutagen dose can either be high or low resulting mutation frequency.

RESOURCES AND METHODS

Planting materials :

In this study three genotypes of garlic IG-2010-3-2, IG-2009-11-1 and Agrifound White were used in this experiment. The cloves of garlic used in the experiment obtained from Indira Gandhi Krishi Vishwavidyalaya, Raipur (C.G.), India were used in the investigation and counted as 40 fresh and big size cloves.

Ethyl methane sulphonate (EMS) induction :

For each treatments cloves were pre-soaked with different concentration like 0.1%, 0.4%, 0.8%, 1.2% and 1.6% on ethyl methane sulphonate (EMS). Cloves soaked in ethyl methane sulphonate (EMS) for 10 days in petriplates and measure germination percentage. After 10 days the treated cloves were grown in open field condition.

Field design and analysis :

Each rows consisted of 6 plots in which 40 cloves per plot were sown with 15 and 10 cm distance between rows and plants, respectively. Data were recorded on germination percentage, Sprouting percentage and LD_{50} (30 DAS) of cloves are determined from the 10^{th} days after the sowing until 30^{th} days. The effective dose (LD_{50}) amount is determined by measuring the dose that decreases the sprouting percentage and survival percentage down to 50%. To determine the effects of ethyl methane sulphonate (EMS) at different concentrations in different genotypes on the values of sprouting percentage, survival percentage and LD_{50} were measured.

The germination percentage at 10 days after soaking in ethyl methane sulphonate (EMS) was then calculated as follows:

$$\text{Germination \%} = \frac{\text{No. of germinated cloves at 10 days after sowing}}{\text{No. of cloves}} \times 100$$

The survival percentage at 10 days after germination was then calculated as follows:

$$\text{Survival \%} = \frac{\text{No. of survival plants at 10 days after sowing}}{\text{No. of cloves}} \times 100$$

The survival percentage at 20 days after germination was then calculated as follows:

$$\text{Survival \%} = \frac{\text{No. of survival plants at 20 days after sowing}}{\text{No. of cloves}} \times 100$$

The survival percentage at 30 days after germination was then calculated as follows:

$$\text{Survival \%} = \frac{\text{No. of survival plants at 30 days after sowing}}{\text{No. of cloves}} \times 100$$

The survival as percentage of control at 20 days (LD_{50}) was then calculated as follows:

$$\text{LD50} = \frac{\text{No. of surival plants at 20 days after sowing}}{\text{No. of germinated plants}} \times 100$$

The survival as percentage of control at 30 days (LD_{50}) was then calculated as follows:

$$\text{LD50} = \frac{\text{No. of surival plants at 30 days after sowing}}{\text{No. of germinated plants}} \times 100$$

OBSERVATIONS AND ANALYSIS

The results obtained from the present study as well as discussions have been summarized under following heads :

Germination percentage :

The data regarding germination percentage of garlic genotype IG-2010-3-2 were differed in different treatments are presented in table 1. The germination percentage decreased with increasing the concentration of EMS as compare to control except 1.2% dose. The germination percentage varied from 20.00% to 45.00%. The maximum germination percentage was recorded in 1.2% dose of EMS (45.00%), followed by 0.1% concentration of EMS (32.50%). Whereas, minimum germination percentage was recorded in 1.6% (20.00%).

The germination % of garlic cloves genotype IG-2009-11-1 recorded for each treatments have been presented in table 2. The germination percentage of garlic cloves differed and ranged from 10.00% to 62.50%. The maximum germination percentage was noted in 0.1% dose of EMS (62.5%). Whereas, minimum germination percentage was noted in 1.6% concentration (10.00%).

Table 1: Germination percentage of genotype IG-2010-3-2

Sr. No.	Treatments	No. of cloves	10 Days after soaking	
			No. of germinated cloves in petriplates	Germination percentage
1.	0.1%	40	13	32.50
2.	0.4%	40	09	22.50
3.	0.8%	40	12	30.00
4.	1.2%	40	18	45.00
5.	1.6%	40	08	20.00
6.	Control	40	09	22.50

Table 2: Germination percentage of genotype IG-2009-11-1:

Sr. No.	Treatments	No. of cloves	10 Days after soaking	
			No. of germinated cloves in petriplates	Germination percentage
1.	0.1%	40	25	62.50
2.	0.4%	40	15	37.50
3.	0.8%	40	12	30.00
4.	1.2%	40	14	35.00
5.	1.6%	40	04	10.00
6.	Control	40	09	22.50

Table 3: Germination percentage of genotype Agrifound White

Sr. No.	Treatments	No. of cloves	10 Days after soaking	
			No. of germinated cloves in petriplates	Germination percentage
1.	0.1%	40	20	50.00
2.	0.4%	40	14	35.00
3.	0.8%	40	14	35.00
4.	1.2%	40	13	32.50
5.	1.6%	40	14	35.00
6.	Control	40	09	22.50

The germination percentage of all the treatments of garlic genotype Agrifound White are given in table 3. The maximum germination percentage was recorded in 0.1% treatment (50.00%). Whereas, minimum germination percentage was recorded in control plot (22.50%).

The reduction or slowing of garlic cloves germination with increased EMS dose may have been caused by a delay or inhibition of physiological processes such as enzyme activity, hormonal imbalance, and inhibition of mitotic process (Kumar and Gupta, 2009; Devi and Mullainathan, 2011; Borovsky *et al.*, 2013). Previous studies in different crops have shown that decreased germination indicates the effectiveness of mutagenic agents (Bahar and Akkaya, 2009).

The different doses of EMS significantly affected the of germination. Increasing the concentration on EMS with decreasing the germination percentage. Similar results about the effect of mutagens have been reported

Table 4: Survival percentage of genotype IG-2010-3-2

Sr. No.	Treatments	No. of cloves	10 DAS		20 DAS		30 DAS	
			No. of survival plants	Survival percentage	No. of survival plants	Survival percentage	No. of survival plants	Survival percentage
1.	0.1%	40	00	0.00	20	50 .00	29	72.50
2.	0.4%	40	00	0.00	18	45.00	33	82.50
3.	0.8%	40	01	2.50	14	35.00	22	55.00
4.	1.2%	40	01	2.50	12	30.00	21	52.50
5.	1.6%	40	00	0.00	08	20.00	15	37.50
6.	Control	40	00	0.00	10	25.00	36	90.00

Table 5: Survival percentage of genotype IG-2009-11-1

Sr. No.	Treatments	No. of cloves	10 DAS		20 DAS		30 DAS	
			No. of survival plants	Survival percentage	No. of survival plants	Survival percentage	No. of survival plants	Survival percentage
1.	0.1%	40	00	0.00	09	22.50	22	55.00
2.	0.4%	40	00	0.00	13	32.50	26	65.00
3.	0.8%	40	00	0.00	15	37.50	20	50.00
4.	1.2%	40	00	0.00	05	12.50	16	40.00
5.	1.6%	40	00	0.00	09	22.50	12	30.00
6.	Control	40	00	0.00	25	62.50	34	85.00

Table 6: Survival percentage of genotype Agrifound White

Sr. No.	Treatments	No. of cloves	10 DAS		20 DAS		30 DAS	
			No. of survival plants	Survival percentage	No. of survival plants	Survival percentage	No. of survival plants	Survival percentage
1.	0.1%	40	00	0.00	16	40.00	32	80.00
2.	0.4%	40	00	0.00	22	55.00	34	85.00
3.	0.8%	40	00	0.00	18	45.00	24	60.00
4.	1.2%	40	00	0.00	19	47.50	27	67.50
5.	1.6%	40	00	0.00	14	35.00	16	40.00
6.	Control	40	01	2.50	18	45.00	23	57.50

Table 7: LD_{50} of genotype IG-2010-3-2:

Sr. No.	Treatments	No. of germinated plants	No. of survival plants 30 DAS	LD_{50} (%)
1.	0.1%	32	29	90.62
2.	0.4%	39	33	84.61
3.	0.8%	25	22	88.00
4.	1.2%	28	21	75.00
5.	1.6%	21	15	71.42
6.	Control	38	36	94.73

in different crops, including cluster bean (Velu *et al.*, 2007), maize (Gnanamurthy *et al.*, 2011), rice (Talebi *et al.*, 2012 b), soybean (Satpute and Fultambkar, 2012), sesame (Anbarasan *et al.*, 2013 (b)), cowpea (Gnanamurthy *et al.*, 2013), pearl millet (Ambli and Mullainathan, 2014) and pigeon pea (Ariraman *et al.*, 2014).

Survival percentage :

The data in Table 4 shows survival percentage of IG-2010-3-2 genotype in different concentration of ethyl methane sulphonate (EMS) at 10, 20 and 30 days after sowing. Results indicated that there were only 1 plant survive out of 40 in 0.8% and 1.2% concentration of EMS in 10 days after sowing therefore survival percentage was 2.50% in 0.8% and 1.2% treatments whereas 20 days after sowing the maximum survival percentage (50.00%) reported in 0.1% concentration and minimum (20.00%) in 1.6% concentration. Data recorded at 30 days after sowing showed the maximum survival percentage was in control plot (90.00%). Whereas, minimum survival percentage in 1.6% treatment (37.50%).

The observation of survival percentage were recorded at 10, 20 and 30 days interval in genotype IG-2009-11-1 (Table 5). Results indicated that there were no germination of cloves after 10 days of sowing therefore survival percentage is 00.00% in each treatments. The maximum survival percentage *i.e.* 62.50% was recorded under control condition followed by 0.8% concentration EMS (37.50%) in 20 days after sowing. The minimum survival percentage (12.50%) was recorded under 1.2% concentration. At 30 days after sowing, the maximum survival percentage (85.00%) was recorded under control plot followed by 0.4% treatment (65.00%).

The data on survival percentage at different stages are presented in Agrifound White (table 6). At 10 days after sowing, the maximum survival percentage was found in control (2.50%). Survival percentage at 20 and 30 days after sowing, it was maximum in 0.4% concentration which is 55.00% and 85.00%, respectively. Whereas, minimum survival percentage was observed under 1.6% EMS treatment 35.00% at 20 day after sowing and 40.00% at 30 days after sowing. The EMS cause random point mutations as Sikora *et al.*, 2011 expressed. As much as the concentration of EMS rises, the probability of point mutation induction would be increased. This mutations may lead to defects in the synthesis of essential compounds for the plant. The higher doses probably would be caused to more genetic injuries on treated plants which may explain why survival rates are lower among of them.

Similarly, Warghat *et al.*, 2011b revealed that sodium azide and gamma rays mutagens decreased the germination and survival percentage of musk okra (*Abelmoschus moschatus*) as compare to control. Jadhav *et al.*, 2012 reported reduction in germination of the okra seed that treated with EMS and gamma rays mutagens. They also reported increase in mortality percentage. Jagajanantham *et al.*, 2013 also noticed that

Table 8: LD_{50} of genotype IG-2009-11-1

Sr. No.	Treatments	No. of germinated plants	No. of survival plants 30 DAS	LD_{50} (%)
1.	0.1%	32	22	68.75
2.	0.4%	29	26	89.65
3.	0.8%	27	20	74.07
4.	1.2%	19	16	84.21
5.	1.6%	20	12	60.00
6.	Control	36	34	94.44

Table 9: LD_{50} of genotype Agrifound White:

Sr. No.	Treatments	No. of germinated plants	No. of survival plants 30 DAS	LD_{50} (%)
1.	0.1%	36	32	88.88
2.	0.4%	37	34	91.89
3.	0.8%	31	24	77.41
4.	1.2%	32	27	84.37
5.	1.6%	27	16	59.25
6.	Control	29	23	79.31

application of EMS and DES mutagens decreased the germination and survival of okra seeds.

LD_{50} :

The LD_{50} of garlic genotype IG-2010-3-2 at different treatments of EMS in survival stage are presented in table 7. The maximum LD_{50} (94.73%) was recorded under control condition at 30 days after sowing followed by 0.1% concentration (90.62%) and 0.8% concentration of EMS (88.00%). The minimum lethal dose 50% (71.42%) was recorded in 1.6% treatment.

It is clear from the table 8 that the LD_{50} of different treatments of EMS consentration of garlic genotype IG-2009-11-1 and ranged from 94.44% to 60.00%. The maximum LD_{50} was recorded in control plot (94.44%). Whereas, minimum LD_{50} was recorded in 1.6% treatment (60.00%). The data regarding LD_{50} recorded in different treatments of EMS in genotype Agrifound White are presented in table 9. The LD_{50} varied from 91.89% to 59.25%. The maximum LD_{50} was recorded in 0.4% treatment (91.89%), followed by 0.1% (88.88%) and 1.2% concentration of EMS (84.37%). However minimum LD_{50} was recorded in 1.6% dose of EMS (59.25%).

Menda *et al.*, 2004 reported that the frequency of M_1 seedlings decreased with increasing EMS concentration. Minoia *et al.*, 2010 reported that Red Setter tomato 1.0% EMS treatment with LD_{49} was more efficient to develop mutant population than 0.7% EMS treatment with LD_{20}. Saito *et al.*, 2011 used different EMS concentrations to develop the Micro-Tom mutant population and 1.0% of EMS with (LD_{63}) treatment represented the most effiecient. While, the LD of Micro-Tom seedlings at 1.0% EMS was lower than that of cv. M_{82}, but higher than cv. Red Setter. The LD observed in our population was higher than 0.7% and lower than that of 1% EMS treated *cv.* Red Setter and 1% EMS treated Micro-Tom (Watanabe *et al.*, 2007).

Conclusion :

In this study, we determined the biological effect of different concentrations of EMS on different genotypes including, IG-2010-3-2, IG-2009-11-1 and Agrifound White on the garlic M_1 generation and its sensitivity to mutagen. We found that the mutagenic effect on various germination behaviors such as germination percentage, survival percentage and LD_{50}. The LD_{50} was detected at about 0.1% EMS in genotype IG-2010-3-2 and Agrifound White, and in genotype IG-2009-11-1 EMS dose 0.4% which is quite high, whereas the LD_{50} was relatively low for 1.6% EMS. The germination percentage and survival percentage showed significant decreases with increasing EMS concentration under all tested presoaking treatments.

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