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Research Article

Duration of Low Temperature Storage, Clove Topping and Smoke Water on Garlic Sprouting and Seedling Vigor

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Abstract

Background and Objective: The extended dormancy period of garlic cloves more than three months after harvest is one of the limiting factors to produce garlic twice a year, in Ethiopia, especially in areas that have a bimodal rainfall distribution. Thus, pre-planting clove treatment is an important agronomic concern for inducing and improving sprouting behavior. This study was aimed to evaluate the effect of low temperature storage, clove topping and smoke water on sprouting behavior of garlic variety ('Tseday').

Materials and Methods: Glasshouse experiment was conducted at Haramaya University to evaluate the effect of cold storage (7°C) durations (10, 20, 30 days and one stored at ambient temperature 21°C), clove topping (whole and cut) and volume by volume concentrations of smoke water to distilled water (0:0, 0:1000, 2:1000, 1:1000, 0.67:1000 and 0.5:1000) on sprouting behavior of improved garlic variety ('Tseday'). The treatments were laid out in a factorial arrangement with three replications using Completely Randomized Design (CRD). **Results:** The interaction effect of 30 days cold stored and topped cloves significantly increased sprouting percentage (46.30-88.42%) over five consecutive measurement days. This treatment resulted in a fourfold increment in the speed of sprouting and increase in pseudostem height (64.34%) and shoot dry mass (46.51%) as compared to the control. However, smoke water treatment showed non-significant effect on all the parameters considered in this particular study. **Conclusion:** The result of the experiment revealed that 30 days of cold storage and topping of garlic cloves could enhance early sprouting and better field establishment.

Key words: Clove topping, dormancy, garlic, sprouting percentage, storage conditions

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Garlic (*Allium sativum* L.) is one of the most important bulb crops and is next to onion (*Allium cepa*) in importance¹. It is widely used as a spice throughout the world. It also has higher nutritive value compared to other bulb crops in addition to containing antibiotics like garlicin and allistatin². It is being consumed for curing many human diseases³.

Garlic is conventionally propagated by its cloves. However, the garlic seed cloves cannot be planted immediately after harvesting because garlic needs a period of dormancy before resuming growth. The period of dormancy varies depending on the variety and storage temperature⁴. Dormancy of garlic creates a problem in use of fresh bulbs for planting and treatments of cloves with different low temperatures and cutting have been reported to have a potential to break dormancy and accelerate sprouting^{5,6}. The optimum storage temperature for sprouting of garlic is in the range between 5 and 10°C⁷. According to Rosa *et al.*⁶ low temperature affects enzymes involved in regulation of sucrose/starch ratio in plants. Sucrose is the free sugar in plant which changes at low temperatures⁸. In such condition, sucrose is catabolized to simple sugars for energy production⁹. Cutting of bulbs are also used to enhance sprouting. Arifin *et al.*⁵ reported that the bulbs received cutting treatments sprouted earlier than whole bulbs in the accessions of shallot and *Allium wakegi*. Rashid and Singh¹⁰ and Rabinowitch and Kamenetsky¹¹ reported that the growing portion of the bulb is topped one-fourth to one-third of the height for easy and fast sprouting. Peter¹² also reported that bulbs are topped to break bud dormancy and enhance uniform sprouting prior to planting.

There has been much research involving improved germination of many species as a result of smoke-stimulated germination. However, the chemical identity of the active compound (s) in smoke and the mode of action still remain a mystery¹³. The treatment of Grand Rapids lettuce seeds with different dilutions of an aqueous smoke extract produces a response curve like phytohormone response curves¹⁴. At high concentrations (dilutions of 1:100 and higher), the smoke extracts are inhibitory to germination, however, lower dilutions (1:1000 dilution) result in significantly increased germination compared to water controls.

Farmers normally use garlic cloves as planting material after long storage period at room temperature that is meant to extend its dormancy to the next cropping year. Thus, it restricts the production cycle to only one per year though two harvests each grown during rain fed and under irrigation during the dry season could be possible. Future prospects of

Ethiopian small and large scale irrigation scheme is to increase vegetable production, including garlic, through increasing the frequency of production cycle per year. Thus, an increase in demand for garlic planting material is expected. To supplement this using freshly harvested garlic clove seed as a planting material after treating with low temperature storage, clove topping or smoke water would be imperative. However, there is scarce information regarding the effects of these treatments on sprouting behavior of garlic varieties. Thus, the objective of this study was to evaluate the effectiveness of low temperature storage, clove topping and smoke water on sprouting behavior of garlic variety ('Tseday').

MATERIALS AND METHODS

The experiment was conducted in a glasshouse at Haramaya University, Ethiopia, to evaluate the effect of cold storage, clove topping and smoke water treatment on sprouting behavior of garlic variety, 'Tseday', which has extended dormancy period of more than three months. The treatments were laid out in factorial arrangement with three replications using Completely Randomized Design (CRD).

Plant material: Freshly harvested garlic bulbs were cured for 10 days (under ambient condition by thinly spreading them on wooden shelves in a diffused light storehouse constructed from wood and having walls netted with a wire mesh and roofed with corrugated iron sheets) and separated into cloves. Medium sized cloves (3.0-3.50 g) were sorted and placed in a refrigerator {MPR-311D (H)} at 7°C such that different samples have received 10, 20 and 30 days of cold storage before planting them in pot placed in a glasshouse. Another set of clove samples were stored at ambient temperature 21°C and serve as the untreated control.

Clove preparation and planting: After cold storage, cloves from each storage treatment were grouped into two, one group topped at $\frac{3}{4}$ length and the second kept whole. These cloves were soaked in volume by volume concentrations of smoke water to distilled water (0:0, 0:1000, 2:1000, 1:1000, 0.67:1000 and 0.5:1000) for 24 h, rinsed for a minute in three changes of sterile water and planted in pots. Smoke water was prepared from *Juniperus procera* by adopting Keeley and Fotheringham¹⁵ method of smoke extraction. The treated and untreated cloves were then planted in pots (12 cloves per pot) filled with top soil, compost and sand (3:2:1), respectively.

Measurement of percent and speed of sprouting: The number of sprouted cloves was recorded starting from the

7th day of planting until the 49th day. Speed of sprouting was estimated with slight modification of the procedure established for estimation of speed of germination by Maguire¹⁶. The numbers of sprouted cloves were counted daily until there was no further sprouting of cloves. An index was calculated by dividing the number of sprouted cloves each day by the number of days in which they have been sprouted, using the following formula.

$$\text{Speed of sprouting} = \frac{N1}{C1} + \frac{N2}{C2} + \dots + \frac{NF}{CF}$$

Where:

N1 = No. of normal sprouts at first count

N2 = No. of normal sprouts at the second count

NF = No. of normal sprouts at the final count

C1 = Days to the first count

C2 = Days to the second count

CF = Days to the final count

To determine the sprouting percentage in number bases on predetermined days after treatments, it was calculated by using the formula:

$$\text{Sprouting percentage} = \frac{\text{No. of sprouted cloves}}{\text{Total number of cloves}} \times 100$$

Measurement of pseudostem height and shoot dry mass:

Five sprouted cloves were randomly taken and pseudostem height was measured 49 days after planting. Shoot dry mass (g) was determined 60 days after planting. After measuring the fresh weight, the above ground part was dried at 70°C to a constant mass in an oven and dry mass was recorded.

Statistical analysis: Data obtained were subjected to analysis of variance (ANOVA) using the General Linear Model (GLM) of the SAS statistical package version 9.2. All significant pairs of treatment means were compared using the Duncan's Multiple Range Test (DMRT) at 5% level of significance.

RESULTS

Effect of pre-planting treatments on sprouting percentage:

The analysis of variance revealed that the two main factors viz., clove topping and cold storage significantly ($p < 0.01$) influenced sprouting percentage of garlic cloves starting from 7-49 days after planting while smoke water treatment did not significantly influence this trait. Clove topping and cold storage interacted to influence significantly sprouting percentage of garlic cloves up to 31 days after planting but not significantly influenced beyond this day (37, 43 and 49 days after planting). The other two factors (clove topping × smoke water and cold storage × smoke water) and three factors (clove topping × cold storage × smoke water) interactions did not significantly influenced sprouting percentage of garlic cloves (Table 1).

Topped cloves which were stored at ambient temperature (0 day) showed a significantly higher percentage of sprouting from 7-31 days after planting as compared to cloves non-topped and 10 days cold stored cloves. Similarly, on these days, topped cloves stored for 20 days showed a significantly higher percentage of sprouting as compared to cloves non-topped and cold stored for 30 days (Table 2).

Planting of 30 days cold stored and topped cloves produced significantly highest percent of sprouts from 7-31 days after planting followed by 20 days cold stored and topped cloves. However, cloves that received neither cold storage nor clove topping treatments (control) gave the lowest sprouting percentage followed by a treatment with no clove topping and 10 days of cold storage (Table 2).

The main effect at 37, 43 and 49 days after planting showed that cloves that received topping gave a higher sprouting percentage as compared to whole cloves. Whereas, treating garlic cloves with different duration of low temperature showed differential response on these days. At 37 DAP, planting of cloves stored for 30 days produced significantly highest percent of sprouts followed by 20 days cold stored cloves as compared to the lowest values recorded

Table 1: Mean square from analysis of variance for sprouting percentage of garlic cloves as influenced by clove topping, cold storage and smoke water

Treatments	Clove topping A (1)	Cold storage B (3)	Smoke water C (5)	A × B (3)	A × C (5)	B × C (15)	A × B × C (15)	Error (47)	CV (%)
7 DAP	7296.006**	7683.095**	14.756 ^{ns}	40.348*	3.182 ^{ns}	7.555 ^{ns}	5.240 ^{ns}	14.406	16.43
13 DAP	12038.966**	10973.508**	34.722 ^{ns}	180.684**	15.817 ^{ns}	14.403 ^{ns}	20.190 ^{ns}	32.906	18.35
19 DAP	12812.982**	15143.229**	8.969 ^{ns}	228.105**	30.574 ^{ns}	18.229 ^{ns}	32.118 ^{ns}	27.703	13.59
25 DAP	10279.706**	11891.075**	73.688 ^{ns}	188.400*	8.873 ^{ns}	24.562 ^{ns}	41.023 ^{ns}	47.455	14.84
31 DAP	12656.250**	14605.838**	114.197 ^{ns}	176.826*	25.462 ^{ns}	52.983 ^{ns}	35.236 ^{ns}	62.046	14.38
37 DAP	19677.854**	4304.269**	41.280 ^{ns}	126.671 ^{ns}	44.753 ^{ns}	41.538 ^{ns}	38.323 ^{ns}	56.259	11.65
43 DAP	10706.500**	4199.299**	15.528 ^{ns}	63.496 ^{ns}	54.880 ^{ns}	40.734 ^{ns}	104.777 ^{ns}	62.015	9.74
49 DAP	5729.648**	1982.220**	4.340 ^{ns}	39.062 ^{ns}	21.315 ^{ns}	27.745 ^{ns}	32.889 ^{ns}	49.959	8.12

ns, * and ** non-significant, significant at $p < 0.05$ and $p < 0.01$, respectively. Numbers in parenthesis represent degree of freedom, DAP: Days after planting and CV (%): Coefficient of variation in percent

Table 2: Effect of clove topping × cold storage interaction on sprouting percentage of garlic cloves starting from 7-31 days after planting

Clove type (CT)	Duration of low temperature storage (DLTS, days)		7 DAP	13 DAP	19 DAP	25 DAP	31 DAP
	Whole	0	10	0.00 ^b	0.00 ^b	10.18 ^b	20.37 ^b
	20	30	8.33 ^a	14.81 ^a	18.52 ^a	28.70 ^a	35.65 ^a
	0	10	24.07 ^d	33.80 ^d	38.89 ^d	47.68 ^d	56.02 ^d
	20	30	31.48 ^c	39.81 ^c	49.54 ^c	55.09 ^c	64.81 ^c
Cut	0	10	15.74 ^f	23.61 ^f	25.46 ^f	35.18 ^f	42.13 ^f
	20	30	19.44 ^e	29.17 ^e	32.41 ^e	40.74 ^e	49.07 ^e
	0	10	39.35 ^b	48.61 ^b	61.11 ^b	65.74 ^b	76.85 ^b
	20	30	46.30 ^a	60.18 ^a	73.61 ^a	77.78 ^a	88.42 ^a

Means designated with different letter(s) in columns of each treatment have significant differences according to DMRT at 5% probability level

Table 3: Main effects of clove topping and cold storage on sprouting percentage of garlic cloves starting from 7-49 days after planting

CT	7 DAP	13 DAP	19 DAP	25 DAP	31 DAP	37 DAP	43 DAP	49 DAP
Whole	15.97 ^b	22.11 ^b	29.28 ^b	37.96 ^b	45.37 ^b	52.66 ^b	72.22 ^b	80.67 ^b
Cut	30.21 ^a	40.39 ^a	48.15 ^a	54.86 ^a	64.12 ^a	76.04 ^a	89.47 ^a	93.29 ^a
DLTS (days)								
0	7.87 ^d	11.80 ^d	17.82 ^d	27.78 ^d	33.56 ^d	55.09 ^c	69.91 ^b	79.63 ^b
10	13.89 ^c	21.99 ^c	25.46 ^c	34.72 ^c	42.36 ^c	55.79 ^c	73.38 ^b	81.71 ^b
20	31.71 ^b	41.20 ^b	50.00 ^b	56.71 ^b	66.43 ^b	68.75 ^b	88.42 ^a	91.90 ^a
30	38.89 ^a	50.00 ^a	61.57 ^a	66.43 ^a	76.62 ^a	77.78 ^a	91.67 ^a	94.67 ^a

Means designated with different letter(s) in columns of each treatment have significant differences according to DMRT at 5% probability level

Table 4: Mean square from analysis of variance for speed of sprouting, pseudostem height and shoot dry mass of garlic cloves as influenced by clove topping, cold storage and smoke water

Treatments	Clove topping	Cold storage	Smoke water	A × B × C					
	A (1)	B (3)	C (5)	A × B (3)	A × C (5)	B × C (15)	(15)	Error (47)	CV (%)
Speed of sprouting	25.362 ^{**}	22.172 ^{**}	0.028 ^{ns}	0.107 ^{**}	0.007 ^{ns}	0.009 ^{ns}	0.012 ^{ns}	0.021	7.21
Pseudostem height	41.388 ^{**}	36.295 ^{**}	0.077 ^{ns}	0.195 [*]	0.004 ^{ns}	0.036 ^{ns}	0.025 ^{ns}	0.051	3.44
Shoot dry mass	0.473 ^{**}	0.514 ^{**}	0.001 ^{ns}	0.019 ^{**}	0.001 ^{ns}	0.001 ^{ns}	0.001 ^{ns}	0.002	4.11

ns * and **non significant, significant at p<0.05 and p<0.01, respectively

for ambient temperature (0 day) stored and 10 days stored cloves. However, at 43 and 49 DAP, cloves that received 30 and 20 days cold storage treatment showed similar result which was significantly higher than ambient temperature (0 day) stored and 10 days stored cloves (Table 3).

Effect of pre-planting treatments on speed of sprouting:

The two main factors (clove topping and cold storage) and the interaction of the two highly significantly (p<0.01) influenced speed of sprouting in garlic cloves while smoke water treatment did not significantly influence this trait. The other two factors (clove topping × smoke water and cold storage × smoke water) and three factors (clove topping × cold storage × smoke water) interactions did not significantly influenced speed of sprouting of garlic cloves (Table 4).

Speed of sprouting was significantly higher in topped cloves stored at ambient temperature (0 day) as compared to non-topped and 10 days cold stored cloves. Similarly, topped cloves stored for 20 days showed a significantly higher speed of sprouting as compared to cloves non-topped and cold stored for 30 days.

The greatest speed of sprouting (3.33) was observed for 30 days cold stored and topped cloves followed by 20 days cold stored and topped cloves (2.91). However, cloves that did not receive cold storage and clove topping treatment (control) recorded the lowest speed of sprouting (0.76) (Table 5).

Effect of pre-planting treatments on pseudostem height:

The two main factors (clove topping and cold storage) significantly (p<0.01) influenced pseudostem height in garlic cloves while smoke water treatment did not significantly influence this trait. Clove topping and cold storage interacted to influence significantly (p<0.05) pseudostem height of garlic cloves. The other two factors (clove topping × smoke water and cold storage × smoke water) and three factors (clove topping × cold storage × smoke water) interactions did not significantly influence pseudostem height of garlic cloves (Table 4).

A significantly higher pseudostem height was recorded for topped cloves stored at ambient temperature (0 day) as compared to non-topped and 10 days cold stored cloves. In the same way, a higher pseudostem height was observed for topped cloves stored for 20 days as compared to cloves non-topped and stored for 30 days.

Table 5: Effects of clove topping and cold storage duration interaction on speed of sprouting, pseudostem height and shoot dry mass of garlic

Clove types	Duration of low temperature storage (days)	Speed of sprouting	Pseudostem height	Shoot dry mass
Whole	0	0.76 ^h	5.02 ^h	0.86 ^h
	10	1.19 ^a	5.27 ^a	0.91 ^a
	20	2.03 ^d	6.71 ^d	1.02 ^d
	30	2.40 ^c	7.09 ^c	1.08 ^c
Cut	0	1.62 ^f	6.16 ^f	0.95 ^f
	10	1.87 ^e	6.41 ^e	0.98 ^e
	20	2.91 ^b	7.56 ^b	1.14 ^b
	30	3.33 ^a	8.25 ^a	1.26 ^a

Means designated with different letter(s) in columns of each treatment have significant differences according to DMRT at 5% probability level

The longest pseudostem height (8.25 cm) was recorded from the treatment of 30 days cold stored and topped cloves followed by 20 days cold stored and topped cloves (7.56 cm). Whereas the smallest pseudostem height was observed for cloves stored at ambient temperature (0 day) and non-topped cloves (5.02 cm) (Table 5).

Effect of pre-planting treatments on shoot dry mass: The two main factors (clove topping and cold storage) and the interaction of the two highly significantly ($p < 0.01$) influenced shoot dry mass in garlic cloves while smoke water treatment did not significantly influence this trait. The other two factors (clove topping \times smoke water and cold storage \times smoke water) and three factors (clove topping \times cold storage \times smoke water) interactions did not significantly influence shoot dry mass of garlic cloves (Table 4).

Cloves stored at ambient temperature (0 day) and topped showed a significantly higher shoot dry mass as compared to 10 days cold stored and non-topped cloves. Correspondingly, 20 days cold stored and topped cloves showed a significantly higher shoot dry mass as compared to 30 days cold stored and non-topped cloves.

The highest mean shoot dry mass (1.26 g per plant) was recorded from the treatment of 30 days cold stored and topped cloves followed by 20 days cold stored and topped cloves (1.14 g per plant). The lowest shoot dry mass (0.8 g per plant) was observed for cloves stored at ambient temperature and non-topped (Table 5).

DISCUSSION

The present study was carried out to observe the effect of duration of low temperature storage, clove topping and smoke water on sprouting and seedling vigor of garlic. The result revealed that planting of 30 days cold stored and topped cloves is effective in sprouting and seedling vigor as it was shown by their highest sprouting percentage, speed of sprouting, pseudostem height and shoot dry mass.

Percent and speed of sprouting were significantly increased by clove topping and increased duration of low temperature. This could possibly be due to the earlier sprouting of cloves. In agreement with this, Arifin *et al.*⁵ reported that bulbs received cutting treatments sprouted earlier than whole bulbs in all accessions of shallot and *Allium wakegi*. This is due to removal of sprout inhibiting substances contained in the removed scale portion as suggested by Lin and Roberts¹⁷ and Wang and Roberts¹⁸ in Easter lily. The substance inhibiting sprouting in the bulbs of *Allium wakegi* has been proved to be abscisic acid¹⁹. Similarly, Yamazaki *et al.*²⁰ and Teaster *et al.*²¹ also reported that the decrease in endogenous ABA content led to early sprouting.

The increase in percent and speed of sprouting by keeping of cloves at low temperature for a specified time is as a result of early release of clove dormancy^{22,23}. This is presumably due to carbohydrate mobilization after starch hydrolysis during cold temperature exposure²⁴. The mobilization of carbohydrate reserves provides energy for development of leaves and photosynthetic apparatus in bulbous plants and also shortens the sprouting time²⁵. Soluble carbohydrate such as sucrose has been reported as the major associated factor for bulb development²⁶ and carbohydrates have been shown to have a positive association in dormancy breaking of lily bulbs²⁷. Cantwell *et al.*²⁸ also showed that storage of garlic at temperatures of 5-10°C than 25°C had promoted respiration rate during storage and the emergence of seedlings.

Pseudostem height and shoot dry mass also significantly increased by clove topping and increased duration of low temperature storage. This is ascribed to the removal of abscisic acid contained in the removed portion that enabled proper growth in vegetative parts. Dutcher and Powell²⁹ and Singha and Powell³⁰ who used apple (*Malus domestica* Borkh cv Northern spy) buds, found that abscisic acid inhibited bud break and shoot elongation. In addition, Munns and Cramer³¹ reported that abscisic acid is generally regarded as inhibitor of shoot growth.

The tremendous increase in pseudostem height and shoot dry mass by increased duration of low temperature storage is due to its effect on early sprouting of cloves that enabled emerging seedlings to utilize reserve food in the cloves for easy establishment and further growth and development^{32,33}. The tallest plantlets and the highest shoot dry mass of the cold treated cloves in the present study might be due to early production of gibberellic acid and other growth substances as a result of the cold treatment. In accordance with the present results, Kurtar and Ayan³⁴ also reported that exposure of tulips to low temperature increased the production of gibberellins and auxins, which were necessary for stalk elongation. Siddique and Rabbani³⁵ also found that plantlet height was influenced by low temperature treatments that enhanced tallness of garlic plants. Ade-Ademilua *et al.*³⁶ reported similar finding and stated that cool temperature storage helps to enhance shoot growth, indicating that garlic cloves treated with cold temperature could have enhanced vegetative growth which increased shoot dry mass of the plant.

CONCLUSION, IMPLICATION AND LIMITATIONS

From the present study, it can be concluded that combination of cold storage (7°C) duration for 30 days and clove topping could be used to treat fresh garlic cloves for higher sprouting percentage, speed of sprouting, pseudostem height and shoot dry mass of garlic.

This study implies that pre planting treatment of freshly harvested garlic cloves resulted in earlier sprouting and good seedling performance. Such seedling performance is indicative of further growth and yield success in a field and also it would be imperative for production of garlic twice in a year under rain-fed and irrigation conditions. In addition, the treatments used in this study are effective, simple, non-chemical and having significant effects with low cost particularly for developing countries to break dormancy and accelerating sprouting in garlic. However, since this study is conducted using only one variety and under a fixed cold temperature, it should be repeated using more cultivars with different low temperature levels to establish optimum storage duration that may result in a better shorter time for breaking of dormancy.

SIGNIFICANCE STATEMENTS

Timely availability of well-sprouted garlic seed cloves at the onset of rainfall as well as for production of garlic under irrigation during the dry season is a pre-requisite for attaining proper planting materials which leads to high yields. However,

garlic cloves are usually influenced by extended dormancy period of more than three months after harvest. So that the results obtained from this investigation exhibited influence of clove topping and cold storage on the sprouting and seedling vigor and gave an indication of breaking dormancy in garlic by applying these pre-planting treatments.

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