# Journal of
# Plant Sciences

ISSN 1816-4951

Academic
Journals Inc.

www.academicjournals.com

Journal of Plant Sciences 4 (3): 49-58, 2009
ISSN 1816-4951


# Pre-Planting (Cold) Treatment of *Allium sativum* Cloves Improves its Growth and Yield Under Open Field and Open Shade Conditions

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**Abstract:** The effects of open field and open shade environmental conditions on the growth and yield of untreated and cold treated cloves of *A. sativum* were investigated. Plants from untreated cloves grew higher under open shade, while those from treated cloves grew higher in the open field. Leaf production was influenced under open field condition and not by pre-planting cold treatment of cloves. There was no significant difference in the total leaf area and fresh weight of plants from untreated cloves grown in the open field and those of treated cloves grown in the open shade until day 56 and 70, respectively. Pre-planting cold treatment of garlic cloves enhanced total leaf area, fresh and dry weight of plants under open shade. Plants from treated cloves had better yield (clove/bulb, clove size, clove dry weight and allicin content/clove) than plants from untreated cloves irrespective of the light condition. Results show that pre-planting treatment of garlic cloves did not only enhance dry matter production but also the allicin content of harvested cloves even under open shade conditions. Plants from treated cloves grown in the open field had the best growth while plants of untreated cloves grown under open shade had the worst (50% less than the former). However, plants from treated cloves grown under open shade had very close growth characteristics with plants from untreated cloves grown in the open field. Results show that the cold pretreatment of garlic cloves help to improve its yield and ability to utilize light.

**Key words:** Allicin content, clove size, dry weight, fresh weight, leaf area, leaf initiation

## INTRODUCTION

*Allium sativum* L. commonly called garlic belongs to the family Alliaceae (Kilgori *et al*., 2007). *Allium sativum* is used mainly as spice, seasoning and flavouring of food stuff due to its pungent flavour. Its medicinal value is also well recognized in the control and treatment of hypertension, worms, germs, bacterial and fungal diseases and other ailments (Kilgori *et al*., 2007). The yield of *A. sativum* in cultivation depends on appropriate cultural practices and factors such as the planting date (Bhuiya *et al*., 2003; Rahman *et al*., 2004), temperature (Rahim and Fordham, 2001), light (Del Pozo and González, 2005), moisture (Bhuiya *et al*., 2003) and soil nutrients (Kilgori *et al*., 2007), which affect the plant at different stages of growth.

The growth stages of *A. sativum* include clove sprouting, shoot growth, bulb growth and maturation (Del Pozo and González, 2005). Clove sprouting and emergence are

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controlled mainly by temperature (Takagi, 1990). The early growth stage of *A. sativum* is suited by exposure of cloves to low temperature. Such exposure could be achieved by using controlled temperature chambers such as refrigerators (Del Pozo and González, 2005) or planting in a cool growing period andis essential for proper development of shoot and good yield of bulb (Bhuiya *et al*., 2003). Seed cloves treated either at 5 or 10°C for 15 to 30 days before planting also accelerates initiation, development and maturity of bulbs relative to those of cloves stored at 15 and 20°C (Rahim and Fordham, 2001). Bulb initiation is promoted by previous exposure of cloves to low temperature (Takagi, 1990; Rahim and Fordham, 2001). A combination of higher temperatures and continuous light have also been found to induce bulb formation (Ledesma *et al*., 1997; Lambers *et al*., 1998). During development, the bulb depends on an increase in total soluble carbohydrates which depends on the photosynthetic activity of the leaves, a factor affected by light (Argüello *et al*., 1997). Bulb development in *A. sativum* therefore depends on the adequate translocation of photosynthates from the leaves to the bulb. Warm temperatures have been identified to promote translocation of food reserve to cloves in *A. sativum* (Argüello *et al*., 1997; Kamenetsky *et al*., 2004). Thus, there is need for cooler temperatures to enhance shoot growth (Bhuiya *et al*., 2003) and higher light intensity to enhance bulb growth (Argüello *et al*., 1997).

High light intensity and low temperature seldom exist together under normal conditions as increase in temperature usually corresponds to increase in light intensity (Mohotti and Lawlor, 2002; Saleh, 2005). Due to this complexity, it is necessary to understand the interactive effects of light intensity and temperature on the growth and bulbing of *A. sativum*.

Open shade conditions reflect cooler temperatures and lower light intensities compared to open field conditions. It is expected that in open field, light intensity is high and of better quality enough to boost photosynthetic activities while light intensity is lower under open shade. Soil gets drier in open field while soil water is more conserved under open shade because the temperature in open field is also higher than that under open shade. The physiological responses of plants to open field and open shade conditions seem to be species specific. Ekinci and Terzioglu (1998) observed higher leaf area in *Triticum aestivum* plants grown under open shade as against those under full sunlight, while López *et al*. (2004) observed increase in the leaf area of *Amaranthus hypochondriacus* grown under full sunlight compared to plants grown under open shade. Henry and Thomas (2002) reported higher dry weights of *Abuliton theophrasti* plants grown under open field, compared to plants grown under open shade conditions. Saleh (2005) noticed higher plant height of *Musa sapientum* under shading conditions. Gouinguené and Turlings (2002) recorded reduced production of volatile in corn plants grown under shade compared to those grown under light. Gouinguené and Turlings (2002) further observed that the production of volatile in corn plants was correlated with photosynthetic activity and no volatile were emitted when the plants were placed in the dark. Reduction in bulb size and yield has been observed in bulbous crops such as *Freesia* (Doorduin, 1992) and *Lycoris* (Choi *et al*., 1991) due to shading environmental conditions.

The effects of the differences in open field and open shade environmental conditions on the growth of a plant species such as *A. sativum*, as light and temperature are important factors that affect its growth and development. This study was therefore aimed at determining the effects of open field and open shade environmental conditions on the growth and yield of both untreated and cold treated cloves of *A. sativum*.

## MATERIALS AND METHODS

### Plant Material

Bulbs of *Allium sativum* of the same variety were obtained from a farmer/grocerier in Mile 12 market, Ketu, Lagos, Nigeria. The experiment was carried out at the University of Lagos Biological garden between 30th June to 30th September, 2008.

### Planting Sites

Two different sites at the Biological garden of the University of Lagos, Nigeria were chosen. The first chosen site was an open field that is under direct sunlight and the second site was a shaded site shielded from direct sunlight by a big *Plumera alba* tree, with many branches. The temperature of the open field site ranged from 29.5 to 33.5°C, while the temperature of the open shade site ranged from 26 to 30.5°C. Light intensity of the open air site ranged from 3142 to 11900 Lx while the light intensity of the open shade ranged from 1546 to 9100 Lx.

### Experiment Set-up

Cloves of average weight of 0.4 g were used for the experiment. Forty cloves were put in the refrigerator at 8°C for 15 days while another set of 40 cloves were kept in the cupboard at room temperature. Cloves were sown in nursery baskets according to the method of Kilgori *et al.* (2007) and watered regularly for 14 days. Experiments were set up in tents built with transparent polythene sheet. Jute bags were each filled with 7.56 kg clay loam soil (pH 7.5). Soil height was 20 cm and a circumference of 100 cm. Two seedlings were transplanted into each bag after 14 days of sowing. A batch of bags with seedlings of treated cloves was put under the tent in the open field and another of batch was put under the tent in the open shade. The same was also done with bags with seedlings of untreated cloves. NPK (1-1-1) fertilizer and poultry manure were added to the soils every 2 weeks as recommended by Kakar *et al.* (2002). The soil was watered every other day with equal quantity of water in both open field and open shade sites.

### Growth Analysis

Plants were randomly uprooted from each row every week from 14 days after treatment. Plant height, total leaf area and shoot fresh and dry weights were determined according to the method of Ade-Ademilua and Obalola (2008).

### Allicin Analysis

The allicin content of the bulb was analyzed using method of Itakura *et al.* (2001). The Standard contains 1600 μg of allicin in each tablet which is equivalent to 960 g of fresh garlic that is 1.667 μg $g^{-1}$ of fresh garlic. Average retention time of allicin was 12.761-12.812 min. The percentage of allicin detected in the standard was 20.727%. The quantity of allicin per gram of fresh garlic in sample was calculated as thus:

$$= \frac{(\text{Weight of allicin in standard})\ \mu g\ g^{-1} \times \text{Allicin in sample (\%)}}{\text{Allicin in standard (\%)}}$$

$$= \frac{1.667\ \mu g\ g^{-1} \times \text{Allicin in sample}}{20.727\%}$$

**Statistical Analysis**

The experiment was carried out in three replicates. The results of the replicates were pooled and expressed as Mean±Standard Error (SE). A one-way analysis of variance (ANOVA) was used to analyze the treatments at 5% level of significance (Zar, 1999). Data presented in line graphs with standard error bars.

## RESULTS

The heights of plants from untreated cloves grown under open shade were higher than those in the open field. On the other hand, the heights of plants from treated cloves grown in the open field were higher than those in the open shade. However, there was no significant difference in the height of plants from untreated and treated cloves grown in the open shade from day 56 (Fig. 1).

Plants in the open field had significant higher number of leaves over time than those in the open shade irrespective of the pre-planting state of the mother cloves. The number of leaves of plants in the open field was the same irrespective of pre-planting treatment throughout the period of analyses. A similar result was obtained for plants in the open shade (Fig. 2).

Plants in the open field had significantly larger total leaf area than their counterparts in the open shade. Plants from treated cloves grew higher than their counterparts under the same growth conditions. However, there was no significant difference in the total leaf area of plants from untreated cloves grown in the open field and those of treated cloves grown in the open shade until day 56 (Fig. 3).

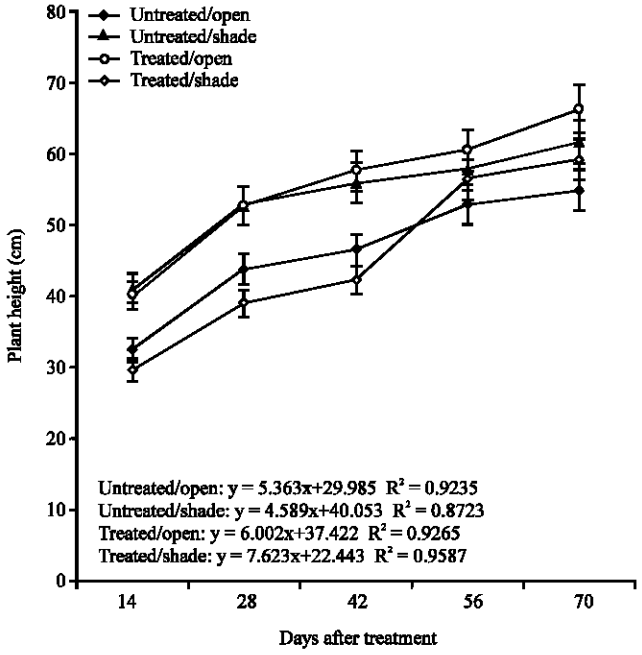


Fig. 1: Height of *Allium sativum* plants from untreated and treated cloves grown under open field and open shade conditions over time. Embedded are equations for each line with the $R^2$ value

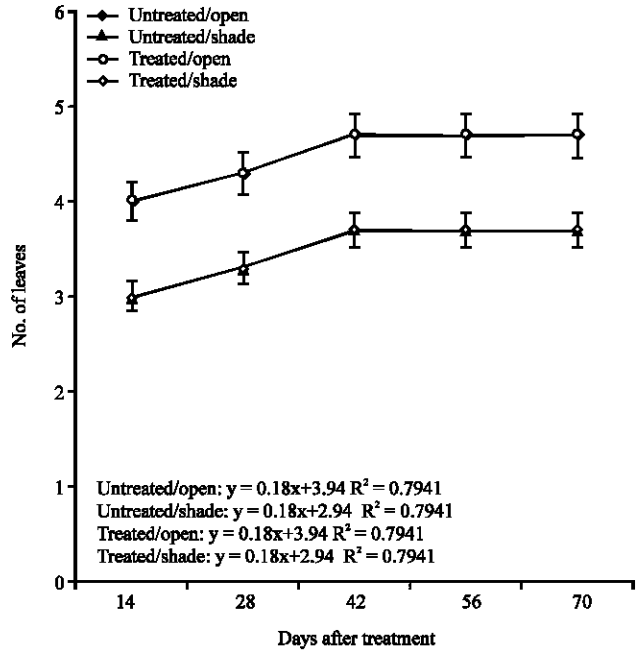


Fig. 2: The number of leaves of *Allium sativum* plants from untreated and treated cloves grown under open field and open shade conditions over time. Embedded are equations for each line with the $R^2$ value

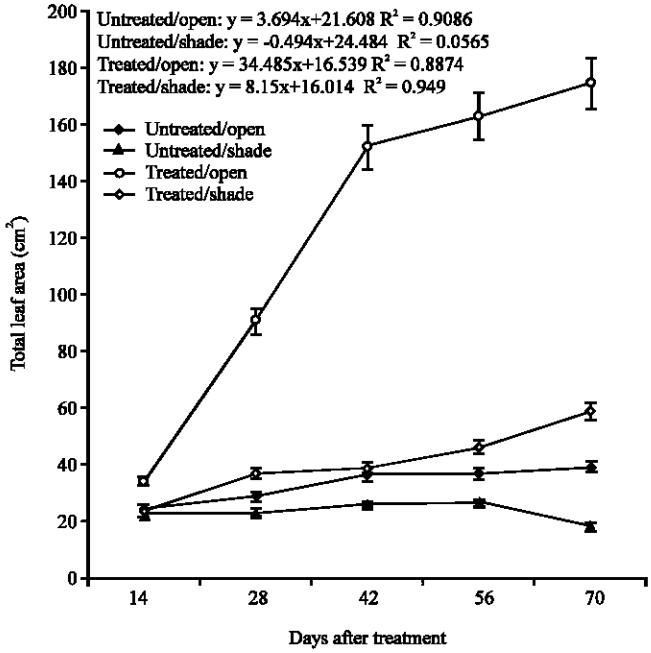


Fig. 3: Total leaf area of *Allium sativum* plants from untreated and treated cloves grown under open field and open shade conditions over time. Embedded are equations for each line with the $R^2$ value

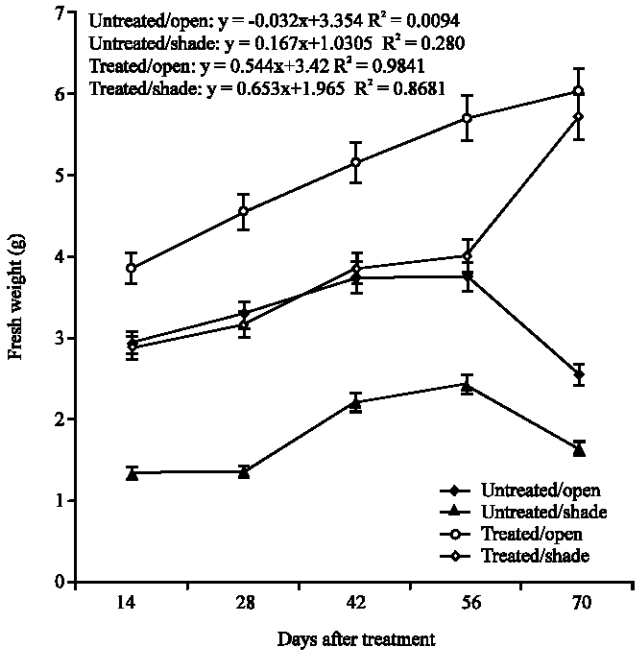


Fig. 4: Fresh weights of *Allium sativum* plants from untreated and treated cloves grown under open field and open shade conditions over time. Embedded are equations for each line with the $R^2$ value

Table 1: Average value of yield parameters of *Allium sativum* plants from untreated and treated cloves grown under open field and open shade conditions

| | Treated cloves | | Untreated cloves | |
|---|---|---|---|---|
| Growth parameters | Open field | Open shade | Open field | Open shade |
| Number of cloves/bulb | 6 | 5 | 4 | na |
| Clove diameter (cm) | 4.92±0.17 | 2.16±0.15 | 1.14±0.08 | na |
| Dry weight of cloves (g) | 0.14±0.03 | 0.08±0.02 | 0.04±0.01 | na |
| Allicin content/clove (μg $g^{-1}$) | 2.26±0.09 | 1.31±0.11 | 0.65±0.10 | na |

na: Not applicable, as the plants in this group did not produce bulbs

Plants from both treated and untreated cloves grown in the open field had significant higher fresh weight than their counterparts in the open shade. However, there was no significant difference in the fresh weight of plants of untreated cloves in the open field and those of treated cloves in the open shade until day 70 (Fig. 4).

Plants from both treated and untreated cloves grown in the open field had significant higher dry weight than plants from similar cloves in the open shade. Plants from treated cloves had significant higher dry weight than plants from untreated cloves most of the time (Fig. 5).

Plants from treated cloves had better yield (clove/bulb, clove size, clove dry weight and allicin content/clove) than plants from untreated cloves irrespective of the light condition. Plants from untreated cloves grown under open shade did not even produce any clove. Plants from treated cloves under open field had better yield that their other counterparts under open shade (Table 1).

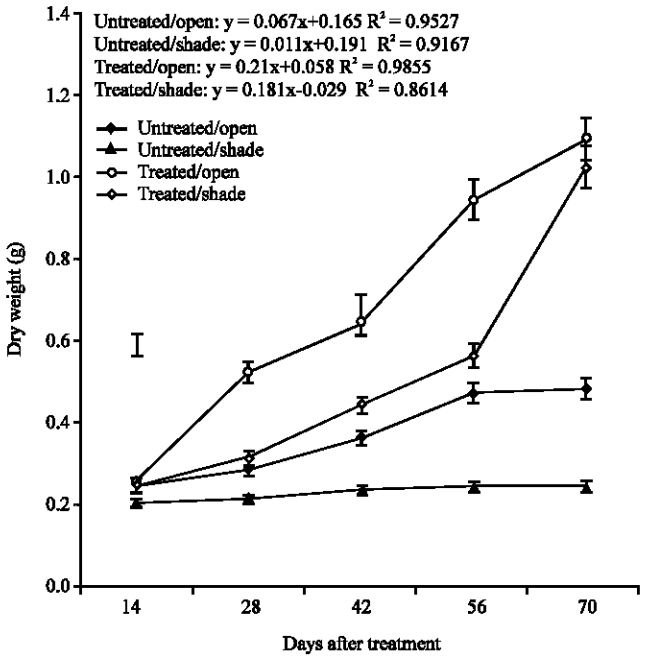


Fig. 5: Dry weights of *Allium sativum* plants from untreated and treated cloves grown under open field and open shade conditions over time. Embedded are equations for each line with the $R^2$ value

Table 2: Comparison of the growth of *Allium sativum* plants from untreated and treated cloves grown under open field and open shade conditions on a scale of 1 to 4

| | Treated cloves | | Untreated cloves | |
|---|---|---|---|---|
| Growth parameters | Open field | Open shade | Open field | Open shade |
| Height | 4 | 3 | 3 | 2 |
| No. of leaves | 4 | 3 | 4 | 3 |
| Total leaf area | 4 | 2 | 3 | 2 |
| Plant fresh weight | 4 | 3 | 3 | 2 |
| Plant dry weight | 4 | 2 | 2 | 1 |
| Yield | 4 | 3 | 2 | 1 |
| Total points | 24 | 17 | 17 | 11 |

The growth of the different groups of plants was compared side to side on a scale of 1-4 based on the increase in plant height, number of leaves/plant, total leaf area, plant fresh weight, plant dry weight and yield over time. Table 2 shows that plants from treated cloves grown in the open field had the best growth while plants of untreated cloves grown under open shade had the worst (50% less than the former). However, plants from treated cloves grown under open shade had very close growth characteristics with plants from untreated cloves grown in the open.

However, cold pre-treatment of the cloves also enhanced yield even under lower light conditions.

## DISCUSSION

The height of plants of untreated cloves under open shade was higher than that of similar plants under open field. Saleh (2005) have shown that *Musa sapientum* plants grow

taller under shading conditions. However, cold treatment of garlic cloves produced an opposite result. Results show that pre-planting cold treatment of cloves does not affect leaf initiation while open field conditions enhances it. Open field conditions induce a higher total leaf area in the garlic plants. Such effect has been observed in *Amaranthus hypochondriacus* by López *et al.* (2004). The results of this experiment showed that cold treatment of garlic cloves enhances total leaf area of plants even under open shade conditions. The fresh and dry weights of garlic plants increased under open field conditions. Henry and Thomas (2002) reported higher dry weights of *Abuliton theophrasti* plants grown under open field, compared to plants grown under open shade conditions. Results showed that cold treatment of garlic cloves enhances the fresh weight and dry matter accumulation of garlic plants. Harvest results showed a reduction in plant yield (clove/bulb, clove size, clove dry weight and allicin content/clove) under open shade conditions. Reduction in bulb size and yield under open shade conditions have been observed in bulbous crops such as *Freesia* (Doorduin, 1992) and *Lycoris* (Choi *et al*., 1991). In the same vein, Gouinguené and Turlings (2002) recorded reduced production of volatile in corn plants grown under shade compared to those grown under light. Reduction in secondary metabolites is linked with reduction in photosynthetic activity under shade conditions. However, the results of this experiment show that pre-planting treatment of garlic cloves did not only enhance dry matter production but the allicin content of harvested cloves even under open shade conditions.

On the overall, results showed that pre-planting treatment of cloves influences both the growth and yield of *Allium sativum* L. under both open field and open shade growth conditions. Low temperature pre-planting of garlic cloves have been shown to enhance the vegetative and bulb growth of the plant (Del Pozo and González, 2005; Rahim and Fordham, 2001). However, this report further shows that cold pre-planting treatment of cloves also enhances the allicin content of cloves and more importantly, results showed that the effect of pre-planting treatment of cloves on the growth and yield of *Allium sativum* L. under open shade conditions is almost at the same level as that of open field growth conditions on untreated cloves. This suggests that pre-planting treatment of cloves could replace the role of open field conditions in influencing the growth and yield of *Allium sativum* L. under open shade conditions. However, further studies need to be carried out to investigate the mechanism through which both pre-planting cold treatment of cloves and open field conditions influence growth and yield of *Allium sativum* L. individually. It would not be surprising to find that the mechanisms are different, since results of this experiment have shown that a combination of pre-planting treatment of cloves and open field growth conditions enhances the growth and yield parameters more than each one of them would.

Garlic is grown for its clove yield (number per bulb, size and allicin content), therefore the result shows that the cold pretreatment of garlic cloves helped to improve its yield and ability to utilize light better. There is a need to investigate the molecular changes caused by cold pretreated of garlic cloves.

Reports have shown that cooler temperatures are required to enhance shoot growth (Bhuiya *et al*., 2003) while higher light intensity help to enhance bulb growth (Argüello *et al*., 1997). Reports of this experiment show that cold pre-treatment of garlic enhanced shoot growth and bulb yield.

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