Tripathi and Singh, 2:2
http://dx.doi.org/10.4172/scientificreports.645

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Research Article

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Hybrid Seed Production in *Helianthus annuus* L. through Male Sterility Induced by Benzotriazole

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Abstract

The effect of various concentrations of benzotriazole ($C_6H_5N_3$) on plant morphology, days taken for first flowering, pollen fertility and yield in *Helianthus annuus* var. MSFH-17 was studied. All the treatments with this chemical hybridizing agent caused reduction in plant height, pollen fertility and yield component. One or two spray of 1% benzotriazole caused insignificant reduction in height and other parameters; however, these treatments induced almost complete pollen sterility among treated plants. On the other hand, the flowering was delayed in benzotriazole treated plants, in comparison to control ones. Plants treated once and twice with 1.0% benzotriazole showed 23.01 and 22.22 g total seed yield/plant, in comparison to 23.68 g total yield/plant produced by control plants. This reduction in yield was insignificant as analyzed by paired student's't' test. The seeds of 1.5% benzotriazole treated plants showed significant enhancement in total yield in F_1 generation from that of control plants due to heterotic effect.

Keywords: Sunflower; Copper chelating compound; Induced male sterility; Phytotoxicity

Introduction

Sunflower enjoys fourth position among oil seed crops, after soybean, rapeseed-mustard and ground nut. In economically important crops, attempts to develop hybrid varieties have been hampered by the lack of a suitable and well-functioning male sterility system. Sunflower is pre-dominantly cross-pollinated; although it has genic and structural male sterility systems, but the commercial hybrid seed usage is very limited, because of the cost of required hybrid breeding. An alternative way for practical and more economical production is to use chemically induced male sterile system for breeding purpose. Various chemisterilants have been used to induce male sterility in a large number of plants for hybrid seed production [1-3]. According to Cross and Schulz (1997), benzotriazole is a copper chelating compound, and act as an inhibitor of microscope development. Tapetum, the target site for benzotriazole action, became metabolically inactive due to presence of degenerated cell organelles, and release large amount of sporopollenin [4,5]. The accumulation of unpolymerized sporopollenin precursors increases the osmotic potential of the locular fluid, and drew water out of the microspores, causing them to collapse [6]. Benzotriazole is found to be quite successful in inducing male sterility in a wide range of plants [4,7,8].

The present communication deals with the effect of benzotriazole ($C_6H_5N_3$) on plant height, days taken for first flowering, pollen fertility and yield component in *Helianthus annuus* L. This study was originally intended to explore the effective dosage of benzotriazole to induce male sterility in sunflower, with least phytotoxic effects.

Materials and Methods

The seeds of *Helianthus annuus* var. MSFH-17 and GK-2002 were sown alternately in a randomized row design pattern, maintaining the 25 cm distance between plants and 45 cm between rows. The plants of var. MSFH-17 were sprayed with different concentrations of benzotriazole and distilled water, and act as female parent and control. On the other hand, plants of GK-2002, left untreated between the rows of treated plants, to facilitate cross pollination. MSFH-17 and GK-2002, both are hybrid varieties released by Mahyco Hybrid Seeds Company Ltd., India and Ganga Kaveri Seeds, India, respectively. The MSFH-17 population have good morphological characteristics and give high yield, but susceptible for fungal diseases. On the other hand, GK-2002 population is resistant for drought and fungal diseases.

Aqueous solutions of different concentrations of benzotriazole (1.0, 1.5 and 2.0% w/v) were prepared in distilled water. One or two drops of liquid soap were added as surfactant into these solutions. Fifty plants (var. MSFH-17) were sprayed about a week before the initiation of floral bud (T_1), and another fifty plants were sprayed twice, first a week before, and second 5 days after floral bud initiation (T_2), individual plants were sprayed with 15 ml of the solution to the runoff. A group of fifty plants were sprayed with distilled water to serve as control.

Pollen fertility was checked at regular intervals with Alexander's Stain [9]. Data on plant height, days taken for first flowering, pollen fertility, total yield/plant, and 100 seed weight from treated and controlled plants were collected and analyzed statistically by paired student's 't' test. The data was collected from the F_1 plants grown from the seeds of 1.5% benzotriazole treated plants, and compared with control and statistically analyzed.

Results

Morphological studies

Plants of *Helianthus annuus* sprayed with different concentrations of benzotriazole displayed reduction in height, and this reduction was gradually increased with the increase in concentration and treatment of benzotriazole (Table 1 and Figure 1). However, plant treated once or twice with 1.0% benzotriazole showed insignificant reduction in height

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Received January 02, 2013; **Published** March 20, 2013

Citation: Tripathi SM, Singh KP (2013) Hybrid Seed Production in *Helianthus annuus* L. through Male Sterility Induced by Benzotriazole. 2: 645 doi:10.4172/scientificreports.645

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from that of control. Leaves of plants sprayed twice with 1.5 and 2.0% benzotriazole showed burning effect and turned brown.

Days taken to first flowering

In benzotriazole treated plants, first flowering was delayed in comparison to control plants (Table 1 and Figure 2). Single treatment with 1.0% benzotriazole caused insignificant enhancement in days taken for first flowering from control plants. Plants treated once or twice with 2.0% benzotriazole took maximum days (69 days) for flowering.

Pollen fertility

Benzotriazole was found to be quite effective in inducing complete pollen sterility in this important oilseed crop (Table 1 and Figure 3). All the concentrations and treatments induced 100% pollen sterility, except single treatment of 1.0% benzotriazole, which induced 97.0% pollen sterility. Sterility induced by benzotriazole was of permanent nature and persistent throughout the flowering period.

Yield

Plants treated once and twice with 1.0% benzotriazole showed insignificant reduction in total yield, along with 97-100% pollen sterility (Table 1 and Figure 4). These plants produced 23.01 and 22.22 g seeds per plant, in comparison to 23.68 g seeds produced by control plants. On the other hand, both the treatments of 1.5 and 2.0% benzotriazole caused significant reduction of yield in treated plants. Seed index (100 seed weight) also showed insignificant reduction with single and double treatments of 1% benzotriazole, which was 5.96 and 5.89 g, respectively, as compared to 6.23 g of that in control plants (Figure 5). The seeds of plants sprayed with higher concentration of benzotriazole were smaller and light weighted.

Performance in F_1 generation

Plants grown from the seeds obtained from 1.0% benzotriazole (T_2) treated plants showed significant vigrosity in vegetative and reproductive parameters (Table 2). The plant height was insignificantly enhanced in F_1 generation, as compared to height in control plants. However, days taken for first flowering and pollen sterility showed insignificant difference in both F_1 and control plants. On the other hand, total yield (124.58%) and 100 seed weight (111.08%) showed significant enhancement over control in F_1 generation plants. This increase in different parameters showed the superiority of F_1 plants over their parents due to heterosis. From the foregoing observations, it is evident that different concentrations of benzotriazole were found to be successful in inducing complete pollen sterility in *Helianthus annuus*. It is interesting to note that one and two spray of 1.0% benzotriazole, along with inducing 97-100% pollen sterility, caused insignificant reduction in yield components. This emphasized its suitability as a successful chemical hybridizing agent for *Helianthus annuus*.

Discussion

Benzotriazole has a toxic effect on plants [10]. Studies have shown that benzotriazole causes morphological changes in tomatoes [11], and has toxic effects on cucumber seedlings and bush beans [12,13] reported that triazoles are visibly toxic to plants, inhibiting root growth and plant development at concentrations greater than 100 mg/L 5-methyl benzotriazole. Benzotriazole has been shown to be an effective nitrification inhibitor [14].

Benzotriazole has also been shown to impact wheat yields *via* chelation of soil copper, causing resulting in male plant sterility and other Cu deficiency symptoms [15]. Benzotriazole act as an inhibitor of microspore development, and thus, causing male sterility in plants. It was also found to be a suitable chemical hybridizing agent for some other economically important crops like *Brassica juncea* [4], *Vicia faba* [7], *Datura alba* [8], *Capsicum annuum*, *Gossypium arboreum* and *Raphanus sativus* [16]. Lower concentration of benzotriazole caused insignificant reduction in yield components among all these crops.

Benzotriazole was not biodegradable, and tend to persist in the environment for a very long time due to their UV stability and resistance to oxidation. These properties of benzotriazole make it an effective CHA for a long time period. Thus, the male sterility induced by benzotriazole was of permanent nature and long lasting. Kaul [12] confirmed the phytotoxicity and stability of benzotriazole in soil solution. Another report by Klingensmith [13] noted that some triazoles, including 5-methyl benzotriazole, may be degraded by plant (*Helianthus annuus*) enzymatic reactions, when present at levels below the phytotoxic threshold. This may lead that although benzotriazole has some phytotoxic properties, but these effects were not found to be effective in sunflower plants, and failed to cause any damage on shoot apices, leaves, buds, etc.

Chemical	Concentrations (%)	Plant height (cm)		Days taken to first flowering		Pollen sterility (%)		Total yield/plant (g)		100 Seed weight (g)	
No. of Treatments		T_1	T_2	T_1	T_2	T_1	T_2	T_1	T_2	T_1	T_2
Benzotriazole	1.0	103.00	101.5	66.0	67.0	97.0*	100*	23.01	22.22	5.96	5.89
		± 3.70 (P=1.40)	± 2.25 (P=1.22)	± 1.86 (P=1.44)	± 2.12 (P=1.53)	± 2.93 (P=105.65)	± 0.0 (P=186.09)	± 1.88 (P=0.75)	± 2.30 (P=1.29)	± 0.15 (P=1.29)	± 0.62 (P=1.75)
	1.5	95.70*	92.20*	67.0	68.0*	100*	100*	21.23*	19.13*	5.02*	4.09*
		± 5.70 (P=5.17)	± 5.30 (P=8.18)	± 3.18 (P=1.71)	± 2.18 (P=4.80)	± 0.0 (P=186.09)	± 0.0 (P=186.09)	± 1.32 (P=3.69)	± 1.89 (P=6.01)	± 0.12 (P=10.63)	± 0.56 (P=29.57)
	2.0	91.34*	89.30*	69.0*	69.0*	100*	100*	17.26*	15.26*	4.10*	3.89*
		± 4.70 (P=9.69)	± 3.74 (P=8.94)	± 2.63 (P=4.93)	± 1.30 (P=6.27)	± 0.0 (P=186.09)	± 0.0 (P=186.09)	± 1.34 (P=14.4)	± 1.92 (P=33.17)	± 0.14 (P=30.66)	± 0.4 (P=25.15)
Control		104.25		65.0		5.25		23.68		6.23	
		± 4.14		± 2.8		± 2.14		± 3.8		± 0.88	

± Standard deviation
Parenthesis represent individual probability (P value) calculated through paired student's't' test.
*P value is greater than significance level (α is 0.05) at 14 degree of freedom (i.e. P<0.05) shows significant change from control group (paired student's 't' test).
Value of significance level (α = 0.05) is 1.761310; Degree of Freedom=14

Table 1: Effect of benzotriazole on vegetative and reproductive parameters of *Helianthus annuus* L.

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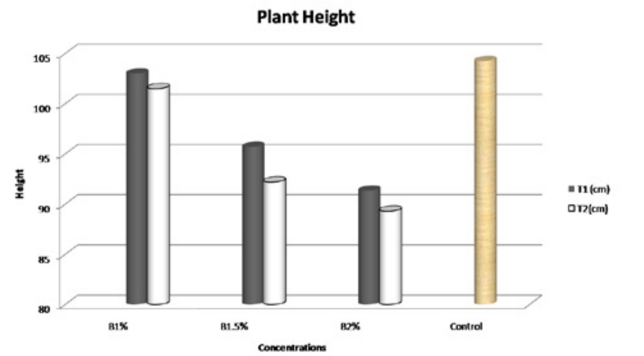

Figure 1: Effect of benzotriazole on plant height.

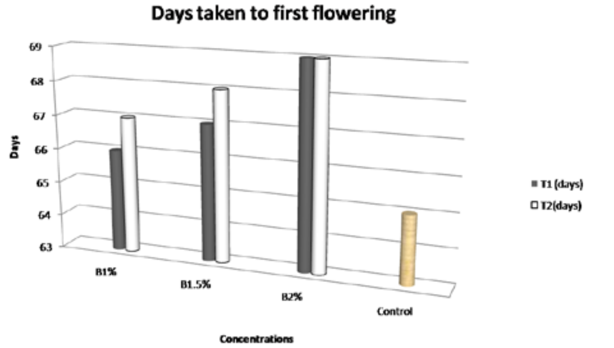

Figure 2: Effect of benzotriazole on days taken to first flowering.

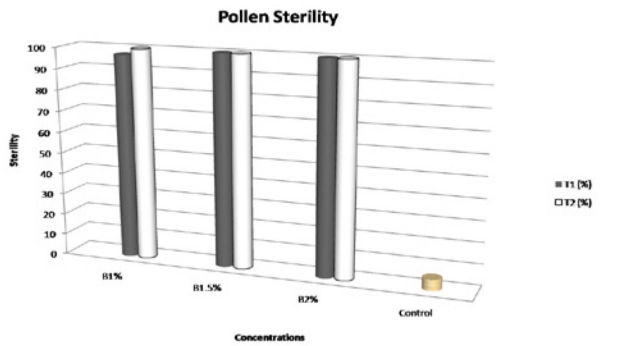

Figure 3: Effect of benzotriazole on pollen sterility.

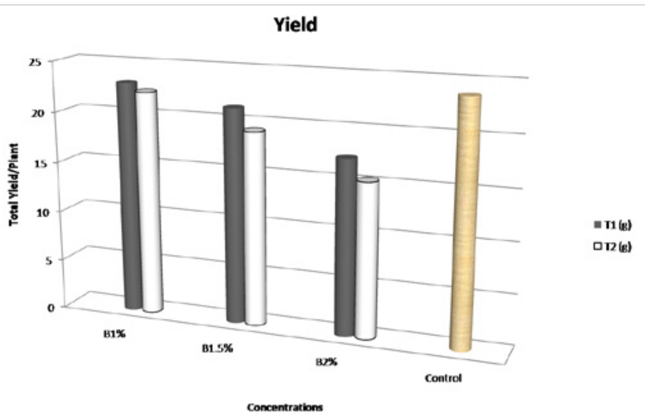

Figure 4: Effect of benzotriazole on total yield/plant.

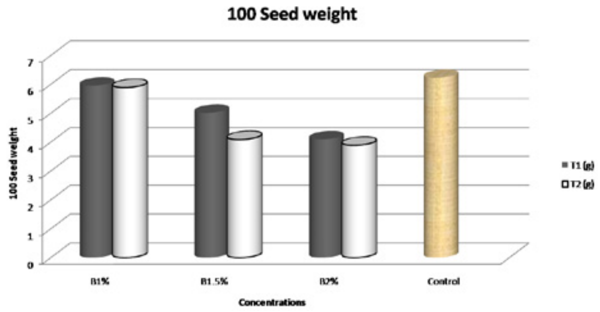

Figure 5: Effect of benzotriazole on 100 seed weight.

Chemical	Plant height (cm)	Days taken to first flowering	Pollen sterility (%)	Total yield/ plant (g)	100 Seed weight (g)
F1 generation of 1.5% benzotriazole treated plants	106.40 ± 2.64 (P=1.64)	66.0 ± 1.49 (P=1.52)	6.85 ± 1.86 (P=1.37)	29.50* ± 1.17 (P=5.08)	6.92* ± 0.21 (P=4.38)
Control	104.25	65.0	5.25	23.68	6.23
	± 4.14	± 2.8	± 2.14	± 3.8	± 0.88

± Standard deviation.
Parenthesis represent individual probability (P value) calculated through paired student's 't' test.
*P value is greater than significance level (α is 0.05) at 14 degree of freedom (i.e. P<0.05) shows significant change from control group (paired student's 't' test).
Value of significance level (α = 0.05) is 1.761310.

Table 2: Performance of F_1 generation plants obtained from the seeds of 1.5% benzotriazole treated plants of *Helianthus annuus* L.

The above observations and discussion conclude that lower dosage of benzotriazole caused poor phytotoxic effects, nearly 100% pollen sterility and insignificant reduction in yield parameters in *Helianthus annuus*. This is also supported by enhancement in yield components of F_1 generation plants over their parent plants, due to heterotic effect. The experiment suggests that benzotriazole was found to be a suitable chemical hybridizing agent for sunflower, and double treatment with 1.0% benzotriazole can be effectively used at commercial scale. The encouraging results from benzotriazole based male sterility call for further evaluation on a larger scale.

Acknowledgements

Dr. Tripathi is grateful to CSIR, New Delhi for the financial assistance in the form of JRF and SRF, and the facilities provided by the Department of Botany, School of Life Sciences, Dr. B.R. Ambedkar University, Agra, India, throughout the period of the investigation, and for the successful completion of the present study as part of his Ph.D. research programme.

References

1. Alexander MP (1980) A versatile stain for pollen, fungi, yeast and bacteria. Stain Technol 55: 13-18.
2. Chauhan SVS, Agnihotri DK (2005) Effect of benzotriazole on pollen fertility and anther development in *Datura alba* L. JPl Sci Res 21: 233-237.
3. Chauhan, SVS, Chauhan, Surabhi (2003) Evaluation of three chemical hybridizing agents in two varieties of broad beans (*Viciafaba* L.). Indian J Genet 63: 128-131.
4. Chauhan, SVS, Kinoshita T (1982) Chemically induced male sterility in angiosperms. Seiken Ziho 30: 54-76.
5. Chauhan SVS, Agnihotri DK, Gupta HK (2005) Efficacy of benzotriazole as a chemical hybridizing agent in chilli (*Capsicum annuum* L.), cotton (*Gossypium arboreum* L.) and radish (*Raphanus sativus* L.). Indian J Genet Plant Breed 65: 223-224.
6. Chauhan SVS, Vandana S (2005) Chemical induction of male sterility in

Brassica juncea (L.) Czern & Coss and its utilization for hybrid seed production. Brassica 7: 117-130.

7. Shivanna KR, Sawahney VK (1997) Pollen biotechnology for crop improvement, Cambridge Univ Press, London, UK.
8. Castro S, Davis LC, Erickson LE (2001) Phytodegradation kinetics of methyl benzotriazole. In: Proceedings of the 2001 Conference on Environmental Research, Kansas State University, Manhattan, USA.
9. Davis D (1954) Benzotriazole, a plant-growth regulator. Science 120: 989.
10. El-Ghazaly GA, Jensen WA (1990) Development of wheat (*Triticum aestivum*) pollen wall before and after effect of gametocide. Can J Bot 68: 2509-2516.
11. Graham RD (1986) Induction of male sterility in wheat using organic ligands with high specificity for binding copper. Euphytica 35: 621-629.
12. Kaul MLH (1988) Male sterility in higher plants. Springer-Verlag, Berlin, Germany.
13. Klingensmith MJ (1961) The effect of certain benzazole compounds on plant growth and development. Am J Bot 48: 40-45.
14. Mc Rae DH (1985) Advances in chemical hybridization. Plant Breed Rev 3: 169-191.
15. Puttana K, Nanje Gowda NM, Prakasa Rao EVS (2001) Regulation of nitrification by benzotriazole, o-nitrophenol, m-nitroaniline and dicyandiamide and pattern of NH_3 emissions from citronella field fertilized with urea. Water Air Soil Pollut 131: 11-17.
16. Singh Vandana, Chauhan SVS (2001) Benzotriazole-a new chemical hybridizing agent for *Brassica juncea* L. J Cytol Genet 2: 81-83.