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Effect of ethrel on lentil (*Lence culinaris* L.) to induce male sterility

Netrapal Singh
Assistant Professor, Department of Botany, LSSSS Government Degree College, Mant, Mathura, Uttar Pradesh, India

Abstract
Present study examined the effect of ethrel on lentil (*Lence culinaris*) to induce male sterility. The term sterility generally includes all those cases that involve failure of production of viable off springs, fruits or seeds. Sterility or barreners becomes most apparent on intra or interspecific hybridization and is essentially a mechanism connected with sexual reproduction in plants. Chemical induction of male sterility has been developed primarily for the production of hybrid crops. The highly competitive hybrid seed market has narrowed the field to a few active agents currently under commercial development. Because prior knowledge of their biochemical action was not required for commercialization, their mechanism are largely unknown.
Chemical hybridizing agent (CHAs) can be used on a large scale for commercial production of hybrid seed. Most CHAs are applied to plants only at a critical stage of male gametophyte development. The aim of this study was to investigate an induce male sterility has been developed by ethrel in lentil. (*Lens culinaris* L.) ethrel known to induce male sterility in some crops and induce pollen sterility in lentil. Foliar application of ethrel brought about complete pollen sterility. This pollen sterility was long lasting. The effect of ethrel treated plants caused almost complete sterility ranging between 97-98.33%.

Keywords: lentil plant growing in garden, ethrel TTC solution FCR solution, light microscope, result

Introduction
Present investigation has been undertaken to study the effect of ethrel on Lentil to induce male sterility. Lentil (Lens culinaris L.) is the most ancient cultivated crops among the legumes. It is indigenous to South Western Asia and the Mediterranean region There is archaeological evidence of lentil., dated back to 7.500 -6.500 BC.
Lentil is one of the early domesticated plant species, as old as those of einkorn, emmer, barley and pea (Harlan, 1992) [12]. The plant was given the scientific name (Lens culinaris L.) in 1787 by Medikus, a German botanist and physician (cubero, 1981; Sehirali, 1988; Hanelt, 2001) [5, 27, 13]. The morphological characteristics of the Lens species as well as synonyms are given by Cubero (1981). The most detailed and complete study of the cultivated lentil was made by Barulina (1930) [4].
Varietal identification was realized by choosing convenient, non-geographical and sometimes utilitarian characteristics. In the diescription, macrosperma has 12 varietis and microsperma has 46 varieties. Lentil in taxonomy is as follows: Kingdome plantae –Plants, Subkingdom Tracheobionta- Vascular plants, Superdivision spermatophyte –Seed plants, Division Magnoliophyta-Flowering plants, Class Magnoliopsida-Dicotyledons, Subclass Rosidae, -Order fabales, -Family Fabaceae, - Pea family, Genus Lens Mill. -lentil, Species Lens culinaris Medik- lentil (Anonymous, 2012).
Lentil (Lens culinaris) is a diploid species 2n=14 belong to the family fabaceae. It is herbaceous annual plant, mostly erect and bushy type with four to six to eight primary branches.
Flower- medianlly zygomorphic, bracteates, bracteolate, bisexual, pedicellate complete pollination cross, pollination by insect. Inflorescence Racemose. Fruits are legume and seeds non-endospermic.
The important lentil –growing countries of the world are India, Canada, Turkey, Bangladesh, Iran, China, Nepal and Syria (Ahlawat,2012). The total cultivated area in the world as around 4.6 million hectares producing 4.2 million tons of seeds with an average production of 1095kg/ha (FAO,2010). The main lentil producers are Armenia, China, Turkey and Croatia.
The term ‘sterility’ generally includes all those case that involve failure of production of viable offsprings, fruits or seeds. Sterility or barreners becomes most apparent on intra or interspecific hybridization and is essentially a mechanism connected with sexual reproduction in plants. The phenomeon of male sterility in bisexual flowering plants was reported as early as the middle of the 18th century by Kolreuter (1763) [14]. The number of studies made by various workers during the past four decades reflect a continuous interest in male sterility (Edwardson, 1970, Laser and Lersten, 1972; Gottschalk and Kaul, 1974; Chauhan and Kinoshita, 1982; Frankel and Galun, 1977; Shivanna and Johri, 1985; Kaul,1988) [8, 17, 6, 9, 18, 16].
Chemical induction of male sterility in plant was first demonstated by Moore (1950) [19] and Naylor (1950) [23]. Various terms such as male sterilant, selective male sterilant, pollen suppressant, pollenocide, androcide, and chemical gametocide, have been used but such terms may not embrace all conceivable modes or site of action.

The terms chemical hybridizing agent (CHAs) was suggested as appropriate (McRae, 1985). Z The cytoplasmic - genetic male sterility is extensively need for commercial production of hybrid seed. CGMs can be successfully utilized for the development of commercial hybrids in both propogated and vegetatively propagated crops. CGMs has been effectively used for commercial production of hybrids to different crops. Chemically induced male sterility is originated due to mutagens or chemical (gametocide) called chemically induced male sterility.
Some gametocides such as NAA, Maleic Hydrazide, Ethrel. c. Male sterility is characterised by nonfunctional Pollen grains, if and where produced, while female gametes function normally.
d. It is the failure of plants to prduce fuctional anthers, pollen, male gametes Absence or atrophy of male organ in a normally bisexual plants. Failure to produce normal sporogenous tissue in stamens.
Inhibition at various stages of pollen development yielding incomplete or imperfect pollen. Ethrel, a source of ethylene, increases the number of pistillate flowers in monoecious cucumbers (McMurray and Miller, 1969; Robinson *et al*.1969) [21] and induces female flowers even on male plant (Mohan Ram and Jaiswal, 1970) [22]. Rowell and miller (1971, 1974) [25, 26] reported upto 100% male sterility in wheat plants treated with 1000-2000 mg-1 ethrel at early, mid and late floral stage, Ethrel did not affect the pistil. Effect of ethrel on barley and wheat show that the effect may range from prevention of normal pollen development to retardation of anther dehiscence. According to Fairey and Stoskopf (1975), the granular from of ethaphon is more effective than the liquid form. In wheat, the application of ethaphone with GA3 (to promote ear emergence) produces sufficient F1 seeds for yield traits. Ethylene has been implicated as a natural regulator of the development and maturation of floral organs. Filaments and corolla growth in flowers of Ipomoea nil are inhibited by ethylene production (Kiss and Koning, 1989) [15]. Different concentration of ethrel induced complete pollen sterility in *Vicia faba* (Chauhan and Chauhan, 2003) [7]. *Brassica Juncea* (Singh and Chauhan, 2004) [28]. *Abelmoschus esculentus* (Agnihotri and chauhan, 2005 and Chauhan and Agnihotri, 2005 C), *Cicer barietinum*, *Lens culinaris*, *Nicotiana tobacum* and *Lycopersicon sesculentum*. (Chauhan *et al*. (2005b).
To study the effect of ethrel on the morphological parameters, pollen fertility and anther development and microsporegenesis in *Capsicum annuum* L. *Lycopersicom esculentum* Mill, and *Nicotiana tobacum* L.
Pollen fertility all the concentrations and number of treatments of ethrel used in present study were effective in inducing complete pollen sterility in *Capsicum annuum*L., *Lycopersicum esculentum* Mill, and *Nicotiana tobacum* L. The pollen sterility was long lasting in all these crops. Complete pollen sterility by ethrel treatment was recorded in all these plants. However, Single spray (T1) of 0.1 % of ethrel induced only 94.9 % in *Cpsicum annuum*, 97.2% in *Lycopersicom esculentum* and 95.7% in *Nicotiana tobacum*. (Agnihotri, D.K. 2008) [3].

Pollen fertility
Pollen fertility is the ability of the pollen to fertilize. There are two tests for pollen fertility.

A. Stainability test
i. TTC TEST
ii. FCR TEST

B. Germination test
i. *In Vitro*

C. Light Microscopic Studies

D. Transmission Electron Microscopy (TEM)

2: The aqueous solution of different concentration (0.1,0.2,0.3 0/0) was made by dissolving it with distilled water.
Number of sprayes. Three sprays of the both the chemicals
Amount of chemicals- 25 ml. of each chemical was used in each spray.

Spray of Chemicals
TTC Test: 1% Tetrazolium chloride solution in 0.15 –Tris HCL buffer at 7.8 ph was used for testing the pollen viability of treated and untreated plants. Thissgive pink / red colour with viable pollen grain and non-viable pollen grain are colourless.

FCR Test: (fluorochromatic Reaction) Heslop-harrion *et al*; (1984)
Take 2-5 ml of sucrose solution in a small glass tube, add drops of stock solution of FAD until the resulting mixture shows persistent turbidity.
The mixture should be used within 30 min. from preparation.
Take drop of sucrose-FAD mixture on a microslide.
Suspend sufficient amount of pollen grains in the drops and ensure uniform distribution of the pollen in preparation.
Incubate the preparation in a humidity chamber (>90RH) for 5-10 min.

At the end of the incubation period, lower a cover glass and observe the preparation the fluorescence microscope with suitable filters.

Transmission Electron Microscopy (TEM)

- For TMT Studies the anthers from freshly collected flowers were cut in to 2-4 small pieces with the help of a sharp blade on a slide in a few drops of 3% glutaraldehyde in 0.1 M phosphate buffer at 6.8 pH.
- These pieces of anthers were fixed in 3% glutaraldehyde in the same buffer for eight hours at room temperature.
- Rinsed 2-3 times in the same buffer.
- Post fixation was done in 1% osmic acidein the same buffer for 4 hours at 40C.
- Dehydration was done in the graded alcohol series for an hour in each step at room temperature.
- Left in 100% ethyl alcohol overnight.
- Transferred to propylene oxide for 30 minutes at 40C making one more change for 30 minutes.
- Transferred to the tubes containing1:1 propylene oxide and epon for overnight in a desiccators. (Epon spurr's Low. Viscosity embedding media) was prepared by gravimeterically adding the following components in a flask: 10 g vinylcyclohexene dioxide; 6g diglyeidyl ether of polypropyleneglycol; 26hg nonenyl succinic anhydride (NSA) and 0.4g dimethylaminoethenol. The mixture was stirred throughly with the help of an electri stirrere.
- Anther pieces were transferred to pure epon and kept in a descicator for 12hours.
- One piece was embedded for one block epon in rubber embedding trays.
- Trays wetre kept in the oven at 40-450 C for 2 days and there after the temperature was raised to 700C.
- Ultra-thin sections (0.75-1.5um) were cut in an automatic ultra-microtome (ultracut E-F) (4) and placed on the grids.
- Sections were stained with uranyl acetate and citrate and observed and photographed with a TEM 100S electron microscope at 80kv. Polaroid (positive as well as negative) film was used for photography.

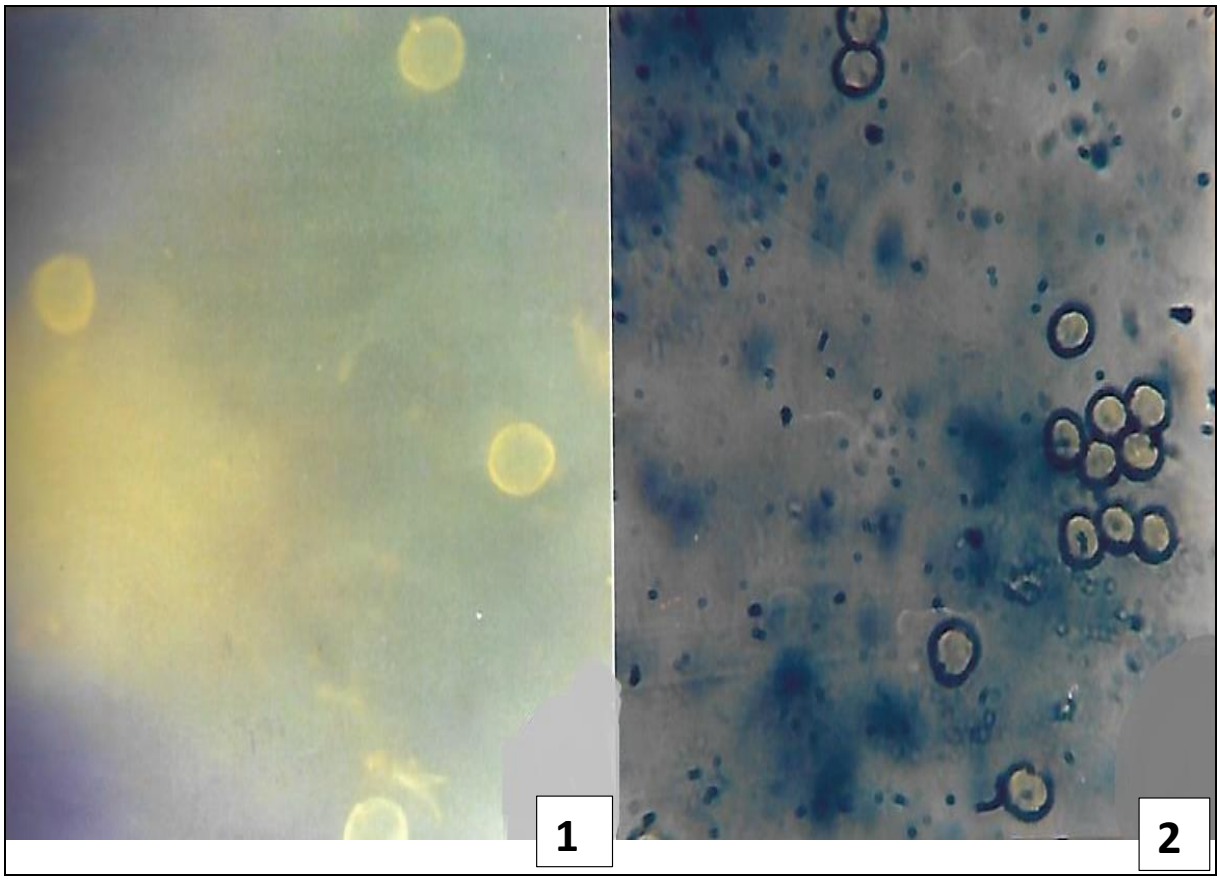

Fig 1-2: Non-viable pollen grains of plants sprayed thrice with 0.3% ethrel checked by FCR and TTC test

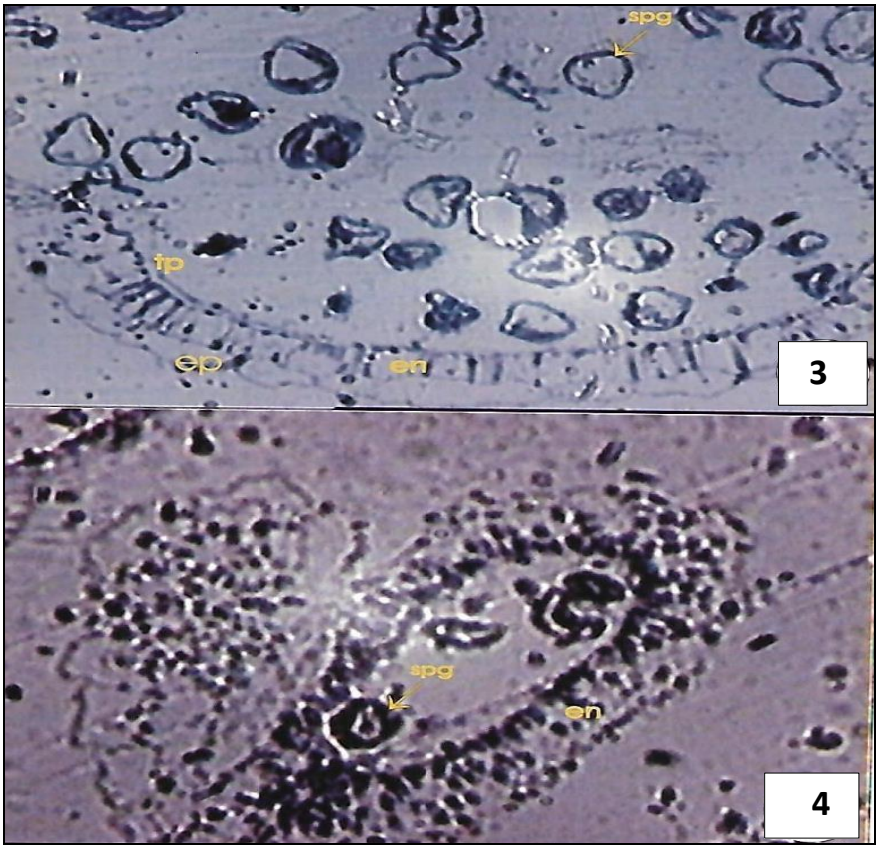

Fig 3-4: T.S. anthers of complete sterile plants treated with 0.3% ethrel

Fig 3 Anther lob at pollen grain stage. showing highly ruptured anther walls. Note the presence of aborted pollen grains (SPG). 1060X.
Fig.4 Anther lobe at pollen grain stage showing complete degeneration of tapetum (TP) and poorly developed endothecium. (EN). Note the presence of degenerated pollen grains (SPG) with scantly cytoplasm.810X.

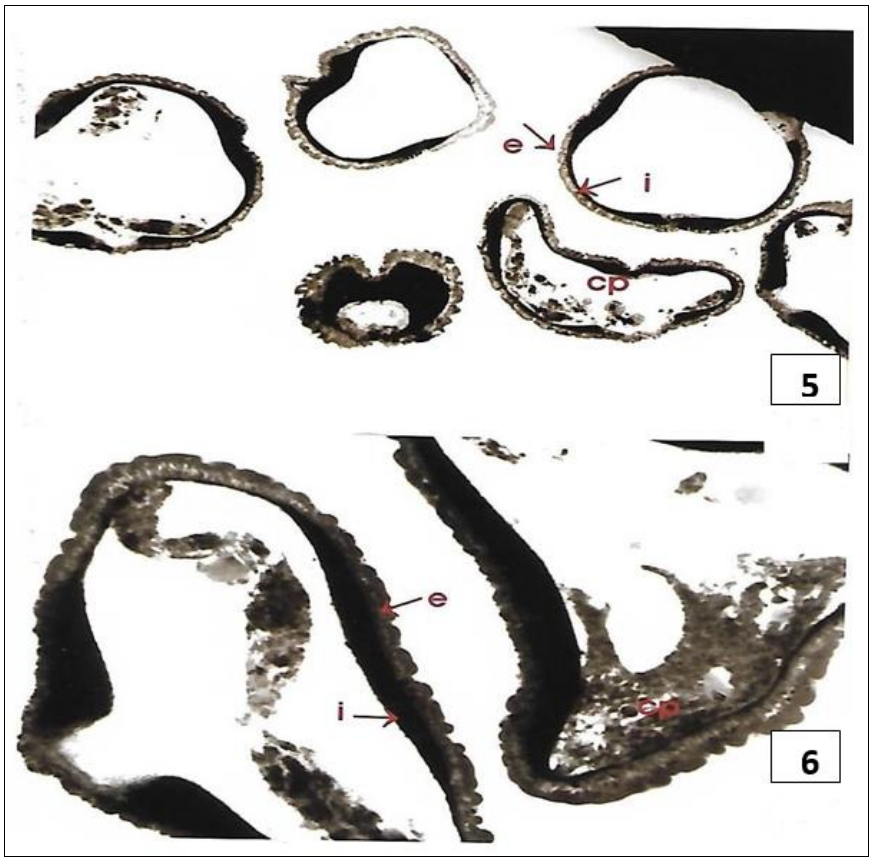

Fig 5-6: TEM Photographs of anthers of plants treated with 0.3% etherel at pollen grain stage.

Fig 5 Non-viable and deformed pollen grains (spg). showing scanty and degenerated cytoplasm (cp). Note the presence of developed exine (e) and intine(i). 730 X.
Fig 6 A magnified view of non-viable pollen grains (spg) exhibiting scanty cytoplasm(cp) with degenerated cell oragane. 1500 X.

Table 1: Effect of different concentrations and number of treatments of ethrel on pollen sterility

Treatment	R1	R2	R3	Mean
Control	2.00	1.00	1.00	1.33
AC1T1	95.00	100.00	100.00	98.33
T2	100.00	100.00	100.00	100.00
T3	100.00	100.00	100.00	100.00
C2T1	100.00	100.00	100.00	100.00
T2	100.00	100.00	100.00	100.00
T3	100.00	100.00	100.00	100.00
C3T1	100.00	100.00	100.00	100.00
12	100.00	100.00	100.00	100.00
T3	100.00	100.00	100.00	100.00
Mean	89.70	90.10	90.10	89.97

Table 2

Means		
Control V/S Treated		
Control	Treated	All
1.33	99.81	89.87

CxT	C1	C2	C3	All
T1	98.33	100.00	100.00	99.44
T2	100.00	100.00	100.00	100.00
T3	100.00	100.00	100.00	100.00
All	99.44	100.00	100.00	99.81

Table 3: Analysis of Variance

Source	DF	SS	MS	F
REPL	2	1.067	0.533	0.590 NS
C	2	1.852	0.926	1.025 NS
T	2	1.852	0.926	1.025 NS
CxT	4	3.704	0.926	1.025 NS
Control v/s treated	1	26186.226	26186.226	28976.561*
Error	18	16.267	0.904	
Total	29	26210.967		

*Significant At 5% Level NS-Not Significant

Table 4; CD for Significant Treatments

Treatments	SE (Mean)	CD At5%
C	0.31688	
T	0.31688	
CxT	0.54885	
Control V/S Treated	0.57854	1.7190

CV. =1.06%

Result

All the tretments of ethrel brought about complete pollen sterility. On the other hand, single spray 0.1% ethrel brought abought 98.33 % sterility.

Ethrel treated plants clearly indicate poorly developed cell organelles in tapetum. Endothecium exhibited feebly developed fibrous thickenings. However, pollen grains of these plants exhibited scantly cytoplasm or cytoplasm with poorly developed cell organelles and degenerated nucleus. Exine of such pollen grains was poorly differentiated.

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