



Full Length Article

Calcium Carbide-induced Changes in Germination, Morpho-phenological and Yield Traits in Cucumber (*Cucumis sativus*)

Muhammad Shakar^{1*}, Muhammad Yaseen¹, Abdullah Niaz², Rashid Mahmood³, Muhammad Mazhar Iqbal⁴ and Tayyaba Naz¹

¹Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad, Pakistan

²Institute of Soil Chemistry and Environmental Sciences, AARI, Faisalabad, Pakistan

³Institute of Agricultural Sciences, University of Punjab, Pakistan

⁴Department of Soil & Environmental Sciences, University College of Agriculture, University of Sargodha, Sargodha, Pakistan

*For Correspondence: shakirsandhu@yahoo.com

Abstract

Calcium carbide (CaC₂) is a well-known source of acetylene and ethylene gases. Its use as a plant growth regulator could be an innovative approach for vegetable production if applied in suitable formulation. Different rates of CaC₂ (10, 20, 30 and 40 mg plate⁻¹) were evaluated regarding seed germination and morphological characteristics under controlled conditions. The results showed that germination rate was significantly improved by using low rates of CaC₂ (10 to 30 mg CaC₂ plate⁻¹) while the highest rate of CaC₂ (40 mg plate⁻¹) suppressed germination rate of cucumber seeds. Likewise, lower rates of CaC₂ were also found effective in enhancing the root and hypocotyl lengths, number of lateral roots and fresh weight of seedling while the highest rate of CaC₂ was proved inhibitory. It was recorded that the CaC₂ induced improvement in seed germination was significantly correlated ($r = 0.75$) with magnitude of ethylene production during imbibition. Moreover, a pot experiment was conducted under natural conditions to select optimum rate and coating material of CaC₂ regarding growth and yield parameters of cucumber. It was found that all rates of CaC₂, irrespective of coating materials, exhibited new primary branches, early female flowering and fruit maturity. Maximum response regarding female flower count, fruit yield and ethylene emission was obtained by the application of paint coated CaC₂ at 300 mg pot⁻¹, which resulted in 34% more fruit yield compared to control plants. © 2016 Friends Science Publishers

Keywords: Acetylene; Ethylene; Calcium carbide; Germination; Cucumber

Introduction

Seed germination is considered as an important mechanism, through which various morpho-physiological changes results in activation of seed embryo. Germination starts with absorption of water, resulting in expansion and elongation of seed embryo and is completed when radicle protrudes from seed covering (Hermann *et al.*, 2007). A large body of literature has been documented regarding the role of plant hormones and their interaction with various environmental factors on seed germination and seedling growth (Kucera *et al.*, 2005; Müller *et al.*, 2006; Hermann *et al.*, 2007). Among various plant hormones, gibberellic acid and ethylene are well known regarding their role in breaking seed dormancy, while ABA is known to cause seed dormancy (Matilla and Matilla-Vazquez, 2008; Linkies *et al.*, 2009). Ethylene is considered to be involved in a wide range of plant activities ranging from seed germination to senescence of plant organs (Arteca and Arteta, 2008). Seed germination is associated with ethylene emission in several plant species including rice, wheat and corn (Zapata *et al.*,

2004). Although, the crucial role of ethylene during seed germination is a well-known fact but the mechanism of action of ethylene is still debatable. Thus, some scientists argue that ethylene is produced in response to germination process, while others oppose that ethylene is mandatory to complete the process of germination (Matilla, 2000; Petruzzelli *et al.*, 2000). In most of the cases, ethylene can stimulate the germination of seeds which may be inhibited due to embryo or coat dormancy, adverse environmental conditions or under the influence of inhibitors (Matilla, 2000).

Ethylene is a gaseous plant hormone involved in regulation of various morphological and physiological processes throughout life cycle of plant including root hair formation, seed dormancy, fruit ripening and numerous abiotic stresses (Matilla, 2000; Kucera *et al.*, 2005). Contrary to the growth inhibitory response, it has also been reported that low concentration of ethylene can stimulate root elongation (Konings and Jackson, 1979), stem elongation (Pierik *et al.*, 2003) and leaf expansion (Fiorani *et al.*, 2002; Khan *et al.*, 2008). Ethylene is also involved in

shoot, root growth and differentiation (Clark *et al.*, 1999; Nicolas *et al.*, 2001) and adventitious root formation (Pan *et al.*, 2002). Ethylene emission from all plant organs has been reported depending on environmental factors and plant species. Moreover, sensitivity and production of ethylene plays essential role regarding sex expression in cucurbits by transition of male buds into female buds and also affect the number of male and female flowers per plant. There is large body of literature regarding the role of foliar-applied source of ethylene (Ethephon) in enhancing femaleness and fruit number of cucumber (Yamasaki *et al.*, 2003; Yu-me, 2009; Thappa *et al.*, 2011), leaf area of mustards (Khan *et al.*, 2008). However, there is a little research regarding the role of biologically substrate-dependent ethylene for cucumber production.

In the past, the effect of liquid CaC₂ based formulation (Retprol) upon tomato and cucumber plants with yield increases up to 70% had been reported (Muromtsev *et al.*, 1993, 1995). Recently, solid calcium carbide (CaC₂) has been shown as a potent source of ethylene which is formed gradually after microbial reduction of acetylene in soil (Yaseen *et al.*, 2006; Kashif *et al.*, 2008). The potential of CaC₂ based formulations has been exploited in improving growth and yield of okra (Kashif *et al.*, 2012), tomato (Siddiq *et al.*, 2012) and sweet pepper (Ahmed *et al.*, 2014). Due to high reactivity of CaC₂ with soil moisture, acetylene and ethylene gases are released rapidly which are prone to loss through diffusion. Therefore, it is needed to coat with non-reactive substances which not only delays the reaction with water but also prolong and maintain its pressure in the soil during critical growth stages of plant. Keeping in view the above facts, a pot experiment was planned to optimize different coating materials for CaC₂ regarding growth and yield characteristics of cucumber.

As, cucumber (*Cucumis sativus* L.) falls into endogenous non-deep physiological dormancy category which is the most common form of physiological dormancy in cultivated crops studied so far (Geneve, 1998). Therefore, cucumber was selected as plant material to investigate if application of CaC₂ could enhance seed germination and seedling growth characteristics. Simultaneously, the effects of CaC₂ on ethylene production were studied in cucumber by using CaC₂ as a source of ethylene under controlled and natural conditions. Moreover, the investigation of different morphological and yield characteristics in response to exogenous application of CaC₂ may be a useful to explore ethylene dependent mechanism in cucumber and the outcome of present study would help in developing CaC₂ as cheaper technology to improve growth and yield in cucumber.

Materials and Methods

Laboratory Experiment

The experiment was conducted with the objective of

optimizing the appropriate level of CaC₂ on the basis of germination and growth response of cucumber. The experiment was carried out under controlled growth conditions in an incubator (Sanyo MIR 253) at 25±1°C with 14 h photoperiod in the laboratory.

Seeds of cucumber cultivar Bolan-F1 (Goreja Seeds®) were sterilized with 70% ethanol and sown in sterilized disposable petri plates. Twenty seeds were placed in each petri plate having filter papers. Holes were made in lids of petri plates; rubber septa were plugged in holes and sealed with silicon gel. Deionized water was injected in petri plates through rubber septum using disposable syringe (5 mL) for watering. Analytical grade CaC₂ (27%) procured from Ningxia National Chemical Group Co. Ltd., China was used. Different rates of CaC₂ (10, 20, 30 and 40 mg CaC₂ plate⁻¹) were spread over round shaped filter paper placed at the bottom of each petri plate. Twenty sterilized seeds were sown on another round shaped filter paper placed above the CaC₂. The seeds were covered with a third round shaped filter paper to control floating and displacement from their original positions after application of deionized water. Each treatment was repeated four times. Seeds were considered to be germinated at the emergence of the radicle and scored. All seed germination related indexes were analyzed at different rates of CaC₂ by subordinate function formulae. The subordinate function values were calculated by using formula of Gower, 1971 as given below:

$$X(u) = (X_i - X_{min}) / (X_{max} - X_{min}) \quad (1)$$

$$\text{Or } X(u) = 1 - (X_i - X_{min}) / (X_{max} - X_{min}) \quad (2)$$

Where: X_u = subordinate function, X_i = Measurement of character of a given treatment, X_{min} = the minimum value of the character among all treatments, X_{max} = the maximum value of the character among all treatments.

Ethylene (C₂H₄) concentrations were determined by using method as described by Khalid *et al.* (2006b) from gas samples collected from petri plates using Gas Chromatograph (Shimadzu GC 2010) fitted with flame ionization detector (FID) and a capillary column (Porapak Q 80-100) operating isothermally under the following conditions: Carrier gas, N₂ (13 mL min⁻¹); H₂ flow rate, 30 mL min⁻¹; Air flow rate, 300 mL min⁻¹; Sample volume 1 mL; Column temperature 70°C. Ethylene concentrations were determined by comparison with reference standards of C₂H₄. The length and width of root and hypocotyl were determined after culturing for 7 days with the help of mm scale and vernier caliper, respectively. At the same time, the fresh weight and number of lateral roots were determined.

Pot Experiment

The pot experiment was conducted in the wire house of Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad-Pakistan during 2009–2010. The wire house has a glass roof with no control over temperature, humidity and light as the sides are open having only a wire net to control birds. During the experimental period, day and

night temperatures in the wire house were $26\pm 5^{\circ}\text{C}$ and $20\pm 5^{\circ}\text{C}$, respectively and average relative humidity was 60.40%. Cucumber nursery plants were grown in thermo pore cups containing media of compost and sand in 1:1 ratio in controlled temperature room. Two seedlings from fifteen days old nursery were transported in earthen glazed pots having 10 kg soil during 1st week of February 2010 and finally one plant was maintained in each pot. The soil was sandy loam having pH of 8.0; total N 0.04%; available P, 5.06 ppm and total organic matter, 0.98%. To stabilize the soil and the indigenous microbial population, the soil was preconditioned 10 days prior to transplanting with one application of Hoagland mineral nutrient solution (one liter, full-strength). The NPK were applied at recommended rate of 100-75-60 kg ha⁻¹ in the form of urea, single super phosphate and murate of potash, respectively. All P and K were applied at sowing time by mixing in soil before pot filling whereas urea was applied in two splits *i.e.*, half at seedling transplantation time by mixing in soil and other half dose at the time of flowering. Calcium carbide was coated with paint, wax, paraffin and gelatin by following the method as described by Mahmood *et al.* (2010). Particles of 2-4 mm diameter of CaC₂ were mixed thoroughly with molten wax, paraffin or paint mechanically in a drum shape container and then mixed with plaster of Paris by gentle manual rubbing to avoid stickiness of particles. Encapsulation was done by filling gelatin capsules with the measured amount of powdered CaC₂. Different rates of each coated CaC₂ (0, 100, 200 and 300 mg kg⁻¹) were placed 6 cm deep in soil in the center of pots after the seedlings were allowed to establish themselves. In control treatments calcium sulphate was added to adjust the amount of calcium added from CaC₂. Canal water treated with Topsin-M at 0.25% was used for irrigation of plants throughout the growth period. After 40 days of seed sowing, ethylene was analyzed by following procedure as prescribed by Atta-Aly (1998). The second developing leaf from the top of the plant was excised 2 mm above the stem surface from each selected plant at 11 a.m. using stainless steel blade. It was placed immediately in 150 mL glass tubes, each containing 5 mL of H₂O for immersing the leaf base in water to prevent drought stress and kept under a 1,000-lux fluorescent light for 8 h. Gas samples (1 mL) were then withdrawn from the glass tube head space and concentrations of C₂H₄ were determined by gas chromatography (Shimadzu-4600, Japan). Different morphological, floral and yield parameters were determined during vegetative and reproductive growth stages of cucumber.

Statistical Analysis

Data were analyzed by using the Statistix 8® computer-based program for computing analysis of variance (ANOVA), while means were compared by Least Significant Difference (LSD) test at 5% level of probability (Steel *et al.*, 1997).

Results

Ethylene Evolution during Germination

Ethylene released from germinating seeds was increased with increasing rates of CaC₂ except the treatment where the highest level of CaC₂ (40 mg plate⁻¹) was applied which significantly reduced the ethylene release (Fig. 1). After 48, 72 and 96 h of incubation, maximum peak of ethylene evolution was observed in the treatment where CaC₂ was applied at 20 and 30 mg plate⁻¹. However, the lowest peak was observed at the highest level of CaC₂ (40 mg plate⁻¹). The application of the highest level of CaC₂ (40 mg plate⁻¹) inhibited the emission of ethylene throughout incubation by 32, 22 and 35% than control at 48, 72 and 96 h of incubation, respectively. A significant correlation coefficient ($r = 0.75$) was found between ethylene emission and germination rate (Fig. 2). Regression equation ($y=10.952x-3.0414$) represents that higher ethylene emission rates result in higher germination rate.

Responses of Seed Germination and Morphological Characteristics of Cucumber to Exogenous Application of CaC₂

Maximum germination rates (94.8% and 93.3%) were recorded in the treatments receiving 20 and 30 mg CaC₂ plate⁻¹, respectively. In comparison with control, germination rate in the treatment of 40 mg plate⁻¹ was decreased by 41.8%. The length of root and hypocotyl of cucumber were increased at low to medium levels while reached maximum by 148.5 and 77.7% of the control, respectively and inhibitory response was observed at the highest rate (40 mg CaC₂ plate⁻¹). However, effect of all rates of CaC₂ on the width of hypocotyl was non-significant ($P \leq 0.05$) except at the highest rate which enhanced the width of hypocotyl maximally. Similarly, number of lateral roots and fresh weight of cucumber seedlings were decreased at the highest rate of CaC₂ (Table 1).

Analysis of the Subordination Function

The correlation of subordination function values of germination and growth indices of cucumber were investigated and found that the correlation coefficients of all studied indices were found significant except the width of hypocotyl (Table 3). According to the analysis of subordination function, application of 30 mg CaC₂ plate⁻¹ was found the most appropriate rate which enhanced the germination and seedling growth of cucumber (Table. 2).

Morphological, Floral and Fruit Yield Responses to Exogenous CaC₂ in Pot Trial

Application rates of 100 to 300 mg pot⁻¹ of each coating material significantly reduced vine length, inter-nodal distance, number of days to flowering, days to fruit maturity while enhanced female flower count, number of nodes on main vine, number of primary branches (Table 4).

Table 1: Effect of different rates of CaC₂ on germination and seedling growth of cucumber

Calcium Carbide (mg plate ⁻¹)	Germination rate (%)	No. of lateral roots	Root length (cm)	Hypocotyl length (cm)	Hypocotyl width (cm)	Seedling weight (g)
0	67.54 c	15.7 d	5.46 d	4.45 d	0.17 b	1.19 d
10	83.73 b	18.34 c	8.21 c	5.66 c	0.17 b	1.33 c
20	93.35 a	21.45 b	10.13 b	6.58 b	0.16 b	1.56 b
30	97.87 a	24.21 a	13.57 a	7.91 a	0.16 b	1.83 a
40	39.23 d	11.23 e	4.02 e	3.89 e	0.22 a	0.93 e

Means sharing same letter in each column are statistically non-significant at P>0.05

Table 2: The Subordination function values for germination and seedling growth of cucumber at different rates of CaC₂

Calcium Carbide (mg plate ⁻¹)	X1	X2	X3	X4	X5	X6	The Subordination function
0	0.48	0.34	0.15	0.13	0.20	0.28	2.80
10	0.75	0.54	0.43	0.44	0.20	0.44	4.66
20	0.92	0.78	0.64	0.66	0.00	0.70	6.12
30	1.00	1.00	1.00	1.00	0.00	1.00	8.00
40	0.00	0.00	0.00	0.00	1.20	0.00	1.20

X1-X6= Germination rate, no. of lateral roots, root length, shoot length, hypocotyl width and fresh seedling weight, respectively

Table 3: Correlation coefficients among germination and growth indices

Indexes	X1	X2	X3	X4	X5	X6
X1	1					
X2	0.971**	1				
X3	0.908*	0.975**	1			
X4	0.911*	0.976**	0.999**	1		
X5	-0.938*	-0.871	-0.747	-0.746	1	
X6	0.937*	0.992**	0.988**	0.987**	-0.821	1

* P<0.05, ** P<0.01

X1-X6= Germination rate, no. of lateral roots, root length, shoot length, hypocotyl width and fresh seedling weight, respectively

Table 4: Effect of different rates and coating materials with CaC₂ on morphological, floral and yield attributes of cucumber during pot study

CaC ₂ rate (mg pot ⁻¹)	Vine length (cm)	Number of primary branches	Number of nodes on main vine	Inter-nodal distance (cm)	Days to first female flower	Days to fruit maturity	Number of fruits per plant	Fruit yield per plant (kg)	Ethylene emission (nL g ⁻¹ h ⁻¹)	Female flower count
Paraffin	165.34 a	4.32 e	23.50 e	7.69 a	52.4 a	13.20 a	13.40 e	1.60 f	0.31 g	20.3 i
100	145.50 b	4.71 de	25.60 de	7.06 b	50.2 ab	12.7 ab	13.77 e	1.69 ef	0.41 ef	22.2 ghi
200	124.50 d	4.8 cd	26.50 cd	6.46c	47.8 bcd	11.4 cde	14.5 cde	1.78 cde	0.47 bc	24.5 cde
300	108.30 f	5.01 bcd	28.40 bc	6.14 de	45.7 bcd	10.01 ef	15.4 bc	1.89 bcd	0.59 a	26.7 ab
Wax										
100	142.40 b	4.81 cd	25.10 de	7.05 b	50.4 ab	11.73 abc	13.81 e	1.70 ef	0.39 ef	22.5 fgh
200	125.40 cd	5.1 bcd	26.50 cd	6.47 c	48.2 bc	10.6 cd	14.4 cde	1.77 cde	0.45 cd	24.1 cdef
300	119.70 f	5.25 ab	29.12 b	6.19 d	45.7 bcd	10.1 f	15.7 bc	1.93 bc	0.58 a	25.8 bc
Paint										
100	137.20 bc	4.71 de	26.40 cd	6.98 b	49.6 abc	11.72 c	15.4 bc	1.93 bc	0.42 de	23.8 defg
200	127.40 cd	5.21 abc	28.50 bc	6.41 c	46.5 bcd	10.45 ef	16.4 ab	2.05 ab	0.51 b	25.3 bcde
300	110.50 ef	5.61 a	32.60 a	6.08 d	43.7 d	9.2 g	17.4 a	2.18 a	0.61 a	27.9 a
Gelatin										
100	142.30 b	4.67 de	25.80 de	7.96 b	49.6 abc	11.82 bc	14.03 de	1.74 def	0.38 f	21.6 hi
200	122.10 de	5.1bcd	26.70 cd	6.49 c	47.2 bcd	10.74 def	15.25 bcd	1.89 bcd	0.46 c	23.7 efg
300	112.50 ef	5.25 ab	29.50 b	6.18 d	45.6 cd	10.1 fg	15.86 b	1.97 b	0.58 a	25.6 bcd
LSD (P<0.05)	11.23	0.43	2.36	0.10	4.16	0.97	1.30	0.16	0.04	1.87

*Values sharing common letter(s) within a column do not differ significantly at P < 0.05 according to LSD test

Maximum response was observed in plants treated with paint coated CaC₂ at 300 mg pot⁻¹. Moreover, differences among various coating materials on CaC₂ were found statistically non-significant regarding morphological and floral attributes. However, maximum fruit yield (2.2 kg plant⁻¹) by cucumber was observed in the treatment where paint coated CaC₂ was applied at 300 mg pot⁻¹,

while minimum fruit yield (1.5 kg plant⁻¹) was observed in control plants (without CaC₂). Application of all treatment significantly affected ethylene evolution, and was the highest with 300 mg pot⁻¹ paint coated CaC₂ (Table 4). Application of 300 mg pot⁻¹ paint coated CaC₂ increased ethylene evolution by 96.7% in cucumber compared to control. Increase in endogenous ethylene

production by the application of various treatments of CaC_2 was found significantly correlated with the increase in female flower count ($R= 0.93$ $p < 0.01$), fruit number ($R= 0.78$ $p < 0.01$) and fruit yield (0.78 $p < 0.01$).

Discussion

Germination rate of cucumber was significantly improved at lower rates of CaC_2 (10 to 30 mg CaC_2 plate⁻¹), while the highest rate of 40 mg CaC_2 plate⁻¹ suppressed germination (Table 1). Root length, number of lateral roots, length and width of hypocotyl and seedling fresh weight are important indicators for determining the growth status of plants. In addition to germination, CaC_2 was also found effective in enhancing root and hypocotyl lengths, number of lateral roots and fresh weight of seedling at lower rates of CaC_2 i.e., 10 to 30 mg plate⁻¹ of CaC_2 . However, the highest rate of CaC_2 i.e., 40 mg plate⁻¹ of CaC_2 showed inhibitory response. These results suggest that the effect of CaC_2 on germination and morphological characteristics is concentration dependent and might follow the biphasic response model, with low rates promoting and high rates inhibiting growth (Pierik *et al.*, 2003). Similar growth promoting effects of ethylene at lower concentration have been reported in *Arabidopsis* (Suge *et al.*, 1997) and Tobacco (Pierik *et al.*, 2003). Application of 400 mg plate⁻¹ of CaC_2 showed triple response along with thickened hypocotyl and reduced hypocotyl and root length. Furthermore, it was found that the CaC_2 induced improvement in seed germination rate was related to its enhancement of ethylene production during imbibition (Fig. 1). Similar results in cucumber had been reported by Lijin (2007) by using ethephon as ethylene source where significant increase in germination rate, germination potential, germination index and activity index than those of control was found. Similarly, improved seed germination in both dormant and non-dormant seeds had been reported in response to the application of ethylene (Kepczynski and Kepczynska, 1997; Matilla, 2000; Kucera *et al.*, 2005). In the present study, enhanced seed germination by the application of CaC_2 was significantly correlated (0.71) with increased ethylene evolution and these results were coincided as reported by Machabee and Saini (1991), and Calvo *et al.* (2004), describing that ethylene was involved in regulation of seed germination. Different peaks of ethylene evolution were observed after the beginning of imbibition in cucumber seeds while ethylene emission was increased with passage of time. Similar results regarding ethylene evolution were reported by Takayanagi and Harrington (1971) in rapeseed in which ethylene evolution was strongly coincided with the rupture of the seed coat, radicle emergence and cotyledon enlargement. Meheriuk and Spencer (1964) also detected ethylene evolution in oat seeds before radicle emergence which was increased with passage of incubation time. In lettuce, Small *et al.* (1993) detected a higher peak of

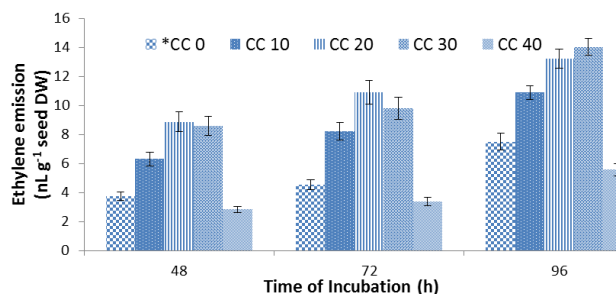


Fig. 1: Effect of different rates of CaC_2 on ethylene evolution from germinating seeds of cucumber during 5 days of incubation period

*CC = CaC_2 (mg plate⁻¹)

Values are means of 4 measurements and bars represent \pm SE (n = 4)

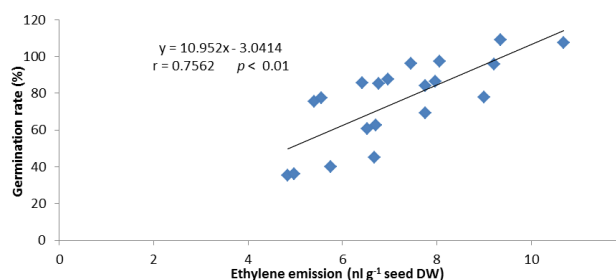


Fig. 2: Relationship between ethylene emission and germination rate in *C. sativus* seeds. Germination rate was plotted as a function of ethylene emission at the end of 96 h incubation period

ethylene evolution after the emergence of radicle, while in some cases main peak of ethylene was found at the time of radicle emergence (Fu and Yang, 1983; Saini *et al.*, 1987).

Differential response of cucumber in terms of morphological, floral and yield parameters due to the application of coated CaC_2 was owing to the specific acetylene and ethylene flux from various rates and coatings of calcium carbide. Ethylene released from CaC_2 reduced plant height, number of days to flowering, days to fruit maturity while improved number of nodes on main vine and number of fruits, resultantly cucumber yield was increased. Reduced plant height, increase in number of primary branches and number of nodes on main vine in response to CaC_2 application is due to classical triple response of plant to ethylene (Frankenberger and Fitzpatrick, 1984) and plant polar auxin transport (Arora *et al.*, 1994).

An increased number of branches in eggplant and pepper have been reported by Miller *et al.* (1969), which induced lateral bud development and damaged the terminal growing bud by the application of ethrel. Inhibition of both cell division and cell elongation has been found with the application of growth retardants, resulting in production of shorter shoots and leaves in melon (Rajala and Peltonen-Saino, 2001). Similar results had been reported by Ouzounidou *et al.* (2008) in melon.

Present study indicated that all rates of CaC₂ irrespective of coating materials had a significant effect on floral characters, which agreed with other research workers who found that foliar application of ethylene (Bhat *et al.*, 2004; Thappa *et al.*, 2011) as well as soil applied ethylene (Shakir *et al.*, 2012; Yaseen *et al.*, 2012) not only enhanced early flowering but also shifted sex expression towards femaleness. Yu-mei (2009) also reported that exogenous ethylene application can obviously inhibit the vine growth, shorten the intermodal length and increase the number of female flowers. Such effects could be attributed to the fact that a lower concentration of CaC₂ slightly inhibited vegetative growth, increased lateral development, reduced respiration (thus increased the carbohydrate levels) and enhanced the development of early pistillate flowers. Such synergistic effects not only increased early fruit setting, but also accelerated the fruit development processes (Abdel-Rahman and Thompson, 1969). Moreover, exogenous ethylene application has been reported to enhance carbohydrate content in cells (Almodares *et al.*, 2013), which in turn promoted pistillate flower production in the vine. It was also noticed that application of CaC₂ reduced inter-nodal distance, thus increased source-sink relationship helped in the early accumulation of photosynthates, necessary for the flowering and fruiting processes of the plant. As the application of CaC₂ consistently supplied ethylene in the proximity of plant that stimulated the developmental processes of cucumber and thus resulting in a higher number of female flowers, then fruits which in turn produced maximum total fruit yield per plant.

Conclusion

According to the analysis of subordination function, the 30 mg CaC₂ plate⁻¹ was found the most appropriate rate which enhanced the germination and seedling growth of cucumber. As the application of CaC₂ consistently supplied ethylene in the proximity of plant roots that stimulated the developmental processes of cucumber and thus resulted in more lateral branches, early female flowering and fruit setting which in turn produced maximum total fruit yield per plant. Moreover, there was a positive significant correlation between endogenous ethylene level and female flowers and all fruit yield characteristics. Among different tested treatments, paint coated CaC₂ at 300 mg pot⁻¹ was found the most appropriate treatment to enhance the fruit number and yield of cucumber plant.

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References

- Abdel-Rahman, M. and B.D. Thompson, 1969. Effect of some growth regulating chemicals on earliness and total yield of cantaloupe and watermelon. In: *Proceedings of Florida State Horticultural Society*, pp: 125–128. University of Florida, Gainesville, USA
- Ahmed, W., M. Yaseen, M. Arshad and M. Shahid, 2014. Effect of polyethylene coated calcium carbide on physiology, photosynthesis, growth and yield of sweet pepper. *Pak. J. Agric. Sci.*, 51: 59–65
- Almodares, A., M. Usofzadeh and M. Daneshvar, 2013. Effect of nitrogen and ethephon on growth parameters, carbohydrate contents and bioethanol production from sweet sorghum. *Sugar Technol.*, 15: 300–304
- Arora, S.K., M.L. Pandita, P.S. Pratap and B.R. Batra, 1994. Response of long melon (*Cucumis melo* var. *utilissimus*) to foliar application of plant growth substances. *Ind. J. Agric. Sci.*, 69: 841–844
- Arteca, R. and J. Arteca, 2008. Effects of brassinosteroid, auxin, and cytokinin on ethylene production in *Arabidopsis thaliana* plants. *J. Exp. Bot.*, 59: 3019–3026
- Atta-Aly, M.A., 1998. Soaking summer squash seeds in low concentrations of cobalt solution before sowing increased plant growth, femaleness, and fruit yield via increasing plant ethylene level. *J. Plant Growth Regul.*, 17: 25–32
- Bhat, K.L., T. Saleem and A. Bushan, 2004. Effect of ethrel, maleic hydrazide and gibberellic acid on growth, yield and quality of watermelon (*Citrullus lanatus* Thumb.). *SKUAST J. Res.*, 2: 221–227
- Calvo, A.P., C. Nicolas, O. Lorenzo, G. Nicolas and D. Rodriguez, 2004. Evidence for positive regulation by gibberellins and ethylene of ACC oxidase expression and activity during transition from dormancy to germination in *Fagus sylvatica* L. seeds. *J. Plant Growth Regul.*, 23: 44–53
- Clark, D.G., E.K. Gubrium, J.E. Barrett, T.A. Nell and H.J. Klee, 1999. Root formation in ethylene-insensitive plants. *Plant Physiol.*, 121: 53–59
- Frankenberger, W.T. Jr. and K.L. Fitzpatrick, 1984. Exogenous hormone production in soil-root systems. In: *Improving Efficiencies in Crop Production Systems, Proceedings of California Plant and Soil Conf.*, pp: 58–61. American Society of Agronomy, USA
- Fu, J.R. and S.F. Yang, 1983. Release of heat pretreatment-induced dormancy in lettuce seed by ethylene or cytokinin in relation to the production of ethylene and the synthesis of 1-aminocyclopropane-1-carboxylic acid during germination. *J. Plant Growth Regul.*, 2: 185–192
- Geneve, R.L., 1998. In: Seed dormancy in commercial vegetable and flower species. D.M. TeKrony (ed.). *Seed Technol.*, 20: 236–250
- Gower, J.C., 1971. A general coefficient of similarity and some of its properties. *Biometrics*, 27: 857–871
- Hermann, K., J. Meinhard, P. Dobrev, A. Linkies, B. Pesek, B. Heß, I. Macháčková, U. Fischer and G. Leubner-Metzger, 2007. 1-Aminocyclopropane-1-carboxylic acid and abscisic acid during the germination of sugar beet (*Beta vulgaris* L.): a comparative study of fruits and seeds. *J. Exp. Bot.*, 58: 3047–3060
- Kashif, S.R., M. Yaseen, M. Arshad and M. Ayub, 2008. Response of okra (*Hibiscus esculentus* L.) to soil given encapsulated calcium carbide. *Pak. J. Bot.*, 40: 175–181
- Kashif, S.U.R., M. Yaseen, H. Raza and A. Kim, 2012. Improving seed germination and green pod yield in okra (*Hibiscus esculentus* L.) using calcium carbide- a new source of ethylene. *J. Plant Nutr.*, 35: 2024–2036
- Khalid, A., M.J. Akhtar, M.H. Mahmood and M. Arshad, 2006b. Effect of substrate-dependent microbial produced ethylene on plant growth. *Microbiology*, 75: 231–236
- Khan, N.A., M.R. Mir, R. Nazar and S. Singh, 2008. The application of ethephon (an ethylene releaser) increases growth, photosynthesis and nitrogen accumulation in mustard (*Brassica juncea* L.) under high nitrogen levels. *Plant Biol.*, 10: 534–538
- Konings, H. and M.B. Jackson, 1979. A relationship between rates of ethylene production by roots and the promoting and inhibiting effects of exogenous ethylene and water on root elongation. *Z. für Pflanzenphysiol.*, 92:385–397

- Kucera, B., M.A. Cohn and G. Leubner-Metzger, 2005. Plant hormone interactions during seed dormancy release and germination. *Seed Sci. Res.*, 15: 281–307
- Lijin, Q., 2007. Effects of Seed Soaking with Ethephon on Seed Germination and Seedling Growth of Cucumber in Greenhouse in Winter. *J. Anhui Agric. Sci.*, 35: 10601
- Linkies, A., K. Müller, K. Morris, V. Turečková, M. Wenk, C.S. Cadman and G. Leubner-Metzger, 2009. Ethylene interacts with abscisic acid to regulate endosperm rupture during germination: a comparative approach using *Lepidium sativum* and *Arabidopsis thaliana*. *Plant Cell*, 21: 3803–3822
- Machabee, S. and H.S. Saini, 1991. Differences in the requirements in the requirement for endogenous ethylene during germination of dormant and non-dormant seeds of *Chenopodium album* L. *J. Plant Physiol.*, 138: 97–101
- Mahmood, R., M. Yaseen, M. Arshad and A. Tanvir, 2010. Comparative effect of different calcium carbide based formulations on growth and yield of wheat. *Soil Environ.*, 29: 33–37
- Matilla, A.J. and M.A. Matilla-Vazquez, 2008. Involvement of ethylene in seed physiology. *Plant Sci.*, 175: 87–97
- Matilla, A.J., 2000. Ethylene in seed formation and germination. *Seed Sci. Res.*, 10: 111–126
- Meheriuk, M. and M. Spencer, 1964. Ethylene production during germination of oat seeds and *Penicillium digitatum* spores. *Can. J. Bot.*, 42: 337–340
- Miller, C.H., R.L. Lower and A.L. Mc Murray, 1969. Some effects of etherel (2 chloroethyl phosphonic acid) on vegetable crops. *HortSci.*, 4: 248–249
- Müller, K., S. Tintelnot and G. Leubner-Metzger, 2006. Endosperm-limited Brassicaceae seed germination: abscisic acid inhibits embryo-induced endosperm weakening of *Lepidium sativum* (cress) and endosperm rupture of cress and *Arabidopsis thaliana*. *Plant Cell Physiol.*, 47: 864–877
- Muromtsev, G.S., O.A. Shapoval, S.V. Letunova and E.V. Druchek, 1995. Effectiveness of the new ethylene producing soil preparation retprol on cucumber plants. *Sel's kokhzyaistvennays-biologiya*, 5: 64–68
- Muromtsev, G.S., S.V. Letunova, L.N. Reutovich, Z.L. Timpanova, I.Y. Gorbatenko, O.V. Shapoval, N.D. Bibik, G.S. Stepanov and D.Y. Druchek, 1993. Retprol —New ethylene releasing preparation of soil activity. *Russ. Agric. Sci.*, 7: 19–26
- Nicolas, I.L., M.A. Echeverria and J.S. Bravo, 2001. Influence of ethylene and Ag⁺⁺ on hypocotyl growth in etiolated lupin seedlings. Effects on cell growth and division. *Plant Growth Regul.*, 33: 95–105
- Ouzounidou, G., P. Papadopoulou, A. Giannakoula and I. Ilias, 2008. Plant growth regulators treatments modulate growth, physiology and quality characteristics of *Cucumis melo* L. plants. *Pak. J. Bot.*, 40: 1185–1193
- Pan, R., J. Wang and X. Tian, 2002. Influence of ethylene on adventitious root formation in mung bean hypocotyl cuttings. *Plant Growth Regul.*, 36: 135–139
- Petruzzelli, L., I. Coraggio and G. Leubner-Metzger, 2000. Ethylene promotes ethylene biosynthesis during pea seed germination by positive feedback regulation of 1-aminocyclo-propane-1-carboxylic acid oxidase. *Planta*, 211: 144–149
- Pierik, R., E.J. Visser, H. de Kroon and L.A. Voeseenek, 2003. Ethylene is required in tobacco to successfully compete with proximate neighbours. *Plant Cell Environ.*, 26: 1229–1234
- Rajala, A. and P. Peltonen-Sainio, 2001. Plant growth regulator effects on spring cereal root and shoot growth. *Agron. J.*, 93: 936–943
- Saini, H.S., P.K. Bassi, J.S. Goudey and M.S. Spencer, 1987. Breakage of seed dormancy of field pennycress (*Thlaspi arvense*) by growth regulators, nitrate and environmental factors. *Weed Sci.*, 35: 802–806
- Shakir, M., M. Yaseen and W. Ahmed, 2012. *Evaluation of Formulated Calcium Carbide on Growth of Cucumber*. LAP Lambert Academic Publishing, Germany
- Siddiq, S., M. Yaseen, M. Arshad and N. Ahmed, 2012. Effect of calcium carbide on photosynthetic characteristics, growth and yield of tomato cultivars. *Pak. J. Agric. Sci.*, 49: 505–510
- Small, J.G.C., C. Schultz and E. Cronje, 1993. Relief of thermo inhibition in 'Grand Rapids' lettuce seeds by oxygen plus kinetin and their effects on respiration content of ethanol and ATP and synthesis of ethylene. *Seed Sci. Res.*, 3: 129–135
- Steel, R.G.D., J.H. Torrie and D.A. Deekey, 1997. *Principles and Procedures of Statistics- A biometrical approach*, pp: 400–428, 3rd edition. McGraw Hill Book Company Inc., New York, USA
- Suge, H., T. Nishizawa, H. Takahashi and K. Takeda, 1997. Phenotypic plasticity of internode elongation stimulated by deep-seeding and ethylene in wheat seedlings. *Plant Cell Environ.*, 20: 961–964
- Takayanagi, K. and J.F. Harrington, 1971. Enhancement of germination rate of aged seeds by ethylene. *Plant Physiol.*, 47: 521–524
- Thappa, M., S. Kumar and R. Rafiq, 2011. Influence of plant growth regulators on morphological, floral, and yield traits of cucumber (*Cucumis sativus* L.). *Kasetsart J.-Nat. Sci.*, 45: 177–188
- Yamasaki, S., N. Fujii and H. Takahashi, 2003. Characterization of ethylene effects on sex determination in cucumber plants. *Sex. Plant Reprod.*, 16: 103–111
- Yaseen, M., M. Arshad and A. Khalid, 2006. Effect of acetylene and ethylene gases released from encapsulated calcium carbide on growth and yield of wheat and cotton. *Pedobiologia*, 50: 405–411
- Yaseen, M., M. Arshad and W. Ahmed, 2012. Effect of calcium carbide dependent release of acetylene and ethylene on nitrification in soil to improve nitrogen use efficiency and yield of vegetables. LAP Lambert Academic Publishing, Germany
- Yu-mei, C., 2009. Effects of ethylene-applying on cucumber growth and development. *J. Anhui Agric. Sci.*, 8: 018
- Zapata, P.J., M. Serrano, M.T. Pretel, A. Amoros and M.A. Botella, 2004. Polyamines and ethylene changes during germination of different plant species under salinity. *Plant Sci.*, 167: 781–788

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