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EFFECTIVENESS OF CALCIUM CARBIDE FORMULATIONS IN EMERGENCE STIMULATION AND GROWTH OF WHEAT (*Triticum aestivum* L.) SEEDLINGS

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Calcium carbide formulations (CCFs) are used for nitrification inhibition and as precursor of ethylene in soil. Their impact on crop growth and yield is thoroughly investigated. However, there is a knowledge gap regarding their influence on seed germination. The present study was planned to study the impact of CCFs on wheat seed germination and early seedling growth. Encapsulated, beeswax coated, paraffin wax coated, paint coated, matrix-1, matrix-2 and matrix-3 CCFs were prepared and applied at 22.5 mg CaC_2 kg^{-1} to sand filled cups. Wheat seeds were sown in sand medium contained in disposable cups. Treatments were allotted according to completely randomized design with three replications. All CCFs except encapsulated and matrix-3 significantly reduced mean emergence time (MET) and increased emergence rate index (ERI) of wheat seedlings. Application of these formulations also improved root growth and increased root: shoot ratio by length; however, influence on root: shoot ratio by weight was found non-significant. Maximum germination and root growth was observed in the treatments of matrix-1 followed by matrix-2, beeswax and paint coated CCFs. To explore the effect of different rates of matrix-1, second experiment was conducted. Its application at 15 mg CaC_2 kg^{-1} was found comparatively better. It is concluded that soil applied CaC_2 in some proper formulation can improve wheat seed germination and root and seedling growth.

Keywords: Calcium carbide formulations, mean emergence time, emergence rate index, ethylene, wheat seedling growth, root: shoot ratio

INTRODUCTION

Ethylene is a plant growth regulator and in addition to other functions, its production in germinating seeds stimulates the germination (Huang and Khan, 1992; Nascimento, 1998). Furthermore, exogenous applications of ethylene or ethephon (an ethylene releasing compound) are reported to remove primary as well as secondary dormancy of seeds sown under optimal or non-optimal conditions (Kerting and Morgan, 1970; Egley, 1980). This indicates that plant seeds have some ethylene-responsive mechanism and their germination inhibition can be completely or partially reversed by exogenous ethylene supply.

Soil applied formulated calcium carbide (encapsulated CaC_2, coated or blended with some hydrophobic material to make matrices) is a known nitrification inhibitor (Patra *et al*., 2006) and an ethylene precursor. In the soil environment it slowly reacts with water to produce acetylene which in turn is reduced to ethylene by soil microbes (Muromtsev *et al*., 1993; Bibik *et al*., 1995). The rate and amount of acetylene and ethylene released from a calcium carbide formulation (CCF) in soil is of practical concern and is strongly influenced by the type of formulation (encapsulated, coated or matrix) and nature and amount of hydrophobic material used in formulation (Freney *et al*., 2000; Mahmood, 2009). A number of researchers prepared a variety of CCFs by using gelatin capsules (Yaseen *et al*., 2006), waxes (Patra *et al*., 2006), polyethylene or enamel paint (Mahmood, 2010) as water repellants. These CCFs were found beneficial regarding their influence on growth and yield parameters of arable crops like wheat (Ahmad *et al*., 2004), rice (Yaseen *et al*., 2005), cotton (Yaseen *et al*., 2006) and others (Siddiq *et al*., 2009). In most of these studies, CCFs were applied after seedling emergence and their impact on seed germination is not yet explored thoroughly. Wheat growth is affected by different factors (Akhtar *et al*., 2012; Ali *et al*., 2012; Hussain *et al*., 2012a, b; Irshad *et al*., 2012). Keeping in view these facts the present study was planned to know the impact of different CCFs on mean emergence time (MET) and root and shoot growth of wheat seedlings.

MATERIALS AND METHODS

This study is comprised of two experiments which were conducted in growth room at Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad, Pakistan.

***Experiment I*:** Different calcium carbide formulations (CCFs), viz. encapsulated (ECC), bees wax coated (BWC), paraffin wax coated (PWC), enamel paint coated (PC), matrix-1 (M1), matrix-2 (M2) and matrix-3 (M3), were compared regarding their influence on seed germination, and root and shoot growth of wheat. The experiment was carried out in disposable plastic cups (each 7 cm long and 5 cm in diameter) by using sieved and three times washed sand as plant growth medium.

***Preparation of calcium carbide formulations*:** On the basis of preparation procedures, CCFs used in the study can be classified broadly as encapsulated, coated and matrices. For encapsulation, weighed amount of powdered CaC_2 (Table 1) was filled into gelatin medical capsules. Calcium carbide dose for one cup sand was filled in one capsule. To prepare coated CCFs calcium carbide (CaC_2) particles (2-4 mm diameter) and coating material – molten beeswax, molten paraffin wax or black enamel paint – were mixed in a rotator mixer in 4:6 ratios, respectively. After mixing, the coated CaC_2 particles were poured out from container and rubbed in plaster of paris to remove surface gluiness. To formulate matrices, powdered (< 200 µm diameter) CaC_2 and plaster of paris (added to provide a bridge for water entry to CaC_2 particles in matrices grains on applying to soil) or paraffin oil were blended with molten polyethylene in a drum shaped container rotating mechanically over flame. Homogenized material was then poured out of container and allowed to cool to room temperature. After cooling matrices clumps were cut to 4-6 mm particles in paraffin oil to block cut ends. The final composition of all seven CCFs is presented in Table 1.

***Experimental plan*:** The experiment was conducted according to completely randomized design (CRD) with three replications. Before sand filling to cups each CCF was placed individually in the cups at 22.5 mg CaC_2 kg^{-1}. Three seeds of wheat cv. Inqulab-91 per cup were sown at 1 cm depth. To assure the same sowing depth in all the treatments, seeds were placed on leveled sand surface in the cups and exactly 1 cm thick sand layer was spread over the seeds. Four cups (12 seeds – 3 per cup) were considered as one experimental unit.

Wheat seedling emergence was observed on daily basis and mean emergence time (MET) and emergence rate index (ERI) were calculated by using formulae MET = $(N_1T_1 + N_2T_2 + \dots N_nT_n)/(N_1 + N_2 + \dots N_n)$ and ERI = S_{te}/MET. Where N_{1-n}, T_{1-n} and S_{te} are the number of seedlings emerged since the time of previous count, number of days after sowing and number of total emerged seedling per experimental unit, respectively.

Fifteen days after sowing, contents of each treatment cup were washed under tap water to remove sand around the seedlings. Moisture over the seedlings was dried in blotting paper folding and fresh biomass weight per $plant^{-1}$ was determined. Each plant was then cut into root and shoot and weights and lengths of both plant parts were recorded. Root to shoot ratios by weight and length were calculated for each plant and means were used in statistical analysis.

***Experiment II*:** On the basis of first experiment results M1 was selected and applied to sand sown wheat seeds in cups at 0, 7.5, 15, 22.5 mg CaC_2 kg^{-1} soil by following the same procedure as described in Experiment I. Emerged seedlings were counted on daily basis and MET and ERI were calculated.

Statistical analysis: The data were subjected to ANOVA and means were compared through LSD test by using Statistix-8.1 computer software package.

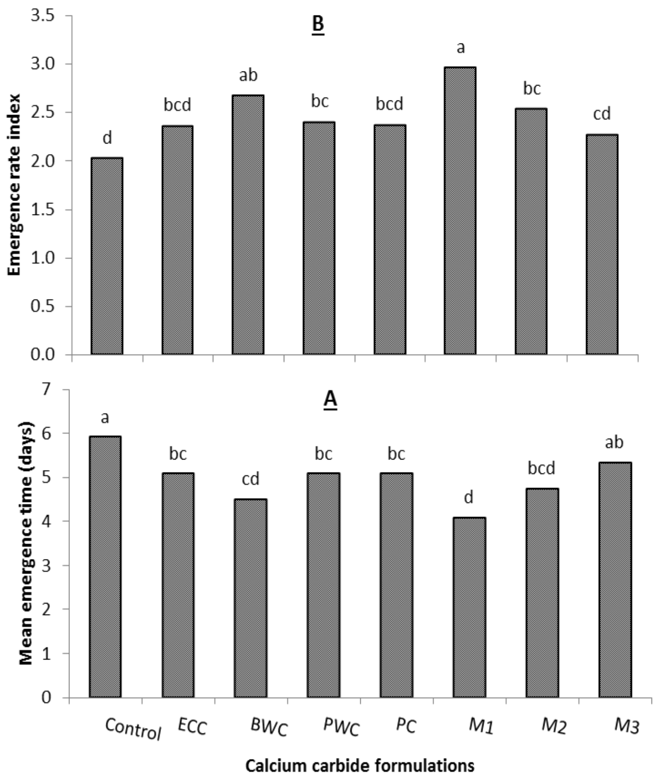
RESULTS AND DISCUSSION

***Mean emergence time and emergence rate index*:** The influence of CaC_2 formulations on MET (days) and ERI of wheat seedlings is presented in Figures 1A and 1B, respectively. It is revealed from the data (Fig. 1A) that CaC_2 application in either formulation, except as M3, significantly reduced the time for wheat seedling emergence. Compared to other CCFs, MET was found minimum with M1 (4.08 days) followed by BWC (4.5 days) and M2 (4.75 days) and

Table 1. Composition and weight (mg) per cup of seven calcium carbide formulations applied at different CaC_2 rates

Calcium carbide formulation	Composition			Weight (mg) of CCF used in one cup at different rates (mg CaC_2 kg^{-1} sand)		
	CaC_2	Hydrophobic material	Other additive	7.5	15.0	22.5
ECC	10.1 mg	Medical capsules	-	3.4	6.8	10.1
BWC	38%	49% BW	13% PP	8.9	17.8	26.6
PWC	37%	50% PW	13% PP	9.2	18.2	27.3
PC	35%	44% Paint	21% PP	9.7	19.3	28.9
M1	21%	58% Polyethylene	21% PP	16.2	32.1	48.1
M2	42%	48% Polyethylene	10% PP	8.1	16.1	24.0
M3	61%	34% Polyethylene	5% PO	5.6	11.1	16.6

ECC – Encapsulated CaC_2; BWC – Beeswax coated CaC_2; PWC – Paraffin wax coated CaC_2; PC – Paint coated CaC_2; M1 – Matrix-1; M2 – Matrix-2; M3 – Matrix-3; BW – Beeswax; PW – Paraffin wax; PP – Plaster of Paris; PO – Paraffin oil; CCF – calcium carbide formulation maximum in control (5.92 days), which was at par with that of the M3 (5.33 days) treatment (Fig. 1A). No statistical difference was observed in effectiveness of PC, PWC and ECC for reducing MET of wheat seedlings.



ECC – Encapsulated CaC_2; BWC – Beeswax coated CaC_2; PWC – Paraffin wax coated CaC_2; PC – Paint coated CaC_2; M1 – Matrix-1; M2 – Matrix-2; M3 – Matrix-3

Figure 1. Effect of calcium carbide formulations on mean emergence time (A) and emergence rate index (B) of wheat seedlings.

Emergence rate index (ERI), another germination related parameter, is a measure of how quickly and uniformly crop seedlings are emerging after sowing, in response to or in the absence of a stimulant or retardant. A high ERI value indicates that seedlings are emerging out more quickly and uniformly, and vice versa. In the present study, ERI of wheat seedlings was significantly increased owing to CaC_2 application in the form of M1, M2, PWC and BWC, whereas in treatments with other CCFs it was statistically at par with that of control (Fig. 1B).

The influence of CCFs on MET and ERI of wheat seedlings seems to be through ethylene, as the production of acetylene and ethylene from the same seven CCFs has already been confirmed in another laboratory experiment (Mahmood, 2009). Involvement of exogenous ethylene in seed germination is a widely accepted fact (Abeles and Lonski, 1969; Huang and Khan, 1992; Nascimento, 1998; Nascimento *et al*., 1999) but the mechanism is poorly understood. However, some possibilities of ethylene involvement in seed germination may be a) by promoting the embryonic hypocotyl cell expansion (Abeles, 1986), b) by lowering the seed water potential and promoting the initiation of growth (Dutta and Bradford, 1994), c) by reducing the inhibitory effects of ABA on germination of seeds (Ketring and Morgan, 1970), d) by decreasing sensitivity of seeds to endogenous ABA (Beaudoin *et al*., 2002) and e) by altering ABA synthesis, as ethylene is a negative regulator of ABA during germination (Ghassemian *et al*., 2002).

Owing to the variations in quality and quantity of hydrophobic material used, all seven CCFs, even applied at the same CaC_2 rate, differed in the elegance of acetylene and ethylene release which influenced MET and ERI of wheat seedlings, accordingly. As M1 was found the best among other CCFs in improving seed germination therefore, it was further investigated by applying at different CaC_2 rates.

The data of 2^{nd} experiment regarding the effect of rate of M1 on MET and ERI of wheat seedlings is summarized in Figures 2A and 2B, respectively.

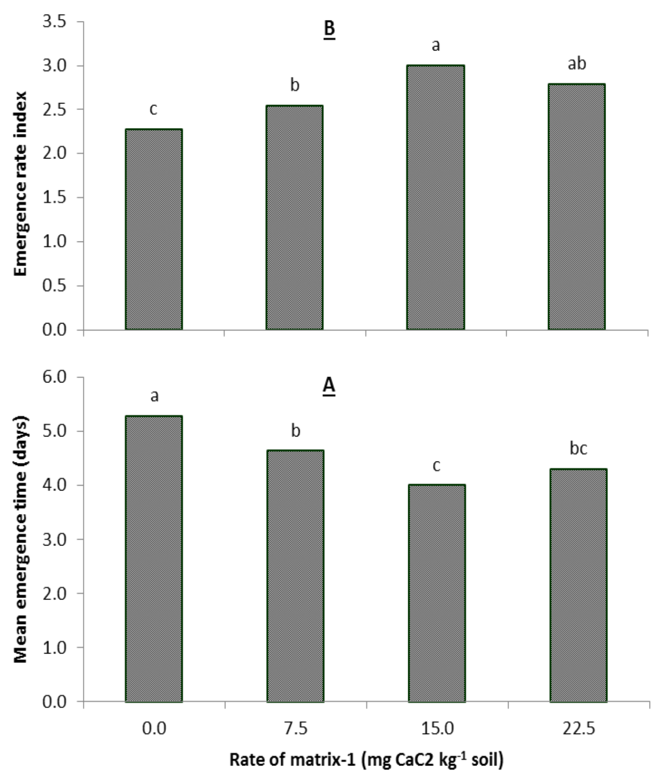


Figure 2. Effect of rate of matrix-1 (mg CaC_2 kg^{-1} soil) on mean emergence time (A) and emergence rate index (B) of wheat seedlings.

Rate of M1 significantly affected the time of wheat seedling emergence. Minimum MET and maximum ERI was noted in the treatment where M1 was applied at 15 mg CaC_2 kg^{-1} soil. In the treatment with M1 at 22.5 mg CaC_2 kg^{-1} soil MET and ERI were statistically at par with that of where it was applied at 15 mg CaC_2 kg^{-1} soil. Lack of reaction of germinating seeds to an extra dose indicates that M1 at 15 mg CaC_2 kg^{-1} soil released sufficient ethylene required for maximum stimulation of wheat seed germination.

***Root and shoot growth*:** Total biomass weight per plant of wheat was significantly increased with M1, M2 and PC formulations (Table 2). However, in treatments with other CCFs, fresh biomass weight of wheat seedlings was statistically at par with that of control (Table 2). Increase in plant biomass weight might be attributed to prompt germination of wheat seeds under the influence of CCFs. Obviously an early emerged plant would have an additional time to grow compared to those which emerged late. This assumption is also supported by the presence of a highly significant and positive correlation (r = 0.68) between total biomass weight and ERI (Table 3).

Excluding ECC and M3, all other CCFs significantly increased root weight of wheat seedlings determined 15 days after sowing (Table 2). Root weight was found maximum and statistically similar in the treatments with M1, M2 and PC. On the other hand compared to control, root length was increased only when CaC_2 was applied as M1 or M2. Root length in treatments with other CCFs was found at par with that of control (Table 1). Owing to calcium carbide application, improvements in root growth of wheat, cotton (Yaseen *et al*., 2006) and Tomato (Siddiq *et al*., 2009 and 2012) are already reported. The influence of CCFs on weight and length of wheat shoots was found statistically non-significant (Table 1).

Data regarding the influence of CCFs on root: shoot ratio by length and weight is presented in Figures 3A and 3B respectively. Calcium carbide application significantly enhanced root: shoot ratio by length, however effect on root: shoot ratio by weight was found statistically non-significant. This indicates that compared to control, CCFs application extended root system at the expense of same amount of photosynthates. Grodzinski and Woodrow (1989) reported a similar shift in root : shoot ratio in ethephon treated tomatoes. They suggested that this shift in carbon partitioning may be caused by a reduction in the dominant carbon sinks. Root growth improvement with ethylene is

Table 2. Effect of calcium carbide formulations on growth parameters of wheat seedlings after fifteen days of sowing

Treatment	Total weight $plant^{-1}$(mg)	Root weight (mg)	Shoot weight (mg)	Root length (cm)	Shoot length (cm)
Control	90 c*	50 d	40	5.2 c	7.0
Encapsulated CaC_2	105bc	59 cd	46	5.6 bc	6.9
Beeswax coated CaC_2	110 bc	64 bc	46	6.5 bc	6.5
Paraffin wax coated CaC_2	110 bc	64 bc	46	5.6 bc	6.1
Paint coated CaC_2	122ab	74 ab	48	6.7 ab	5.9
Matrix-1	133 a	81 a	52	7.9 a	6.1
Matrix-2	130 a	78 a	53	6.3 bc	5.8
Matrix-3	102 bc	53 d	50	5.4 bc	6.2
LSD (0.05)	20.29	10.83	N.S	1.36	N.S

*Means sharing similar letters in a column do not differ significantly at $p \leq 0.05$

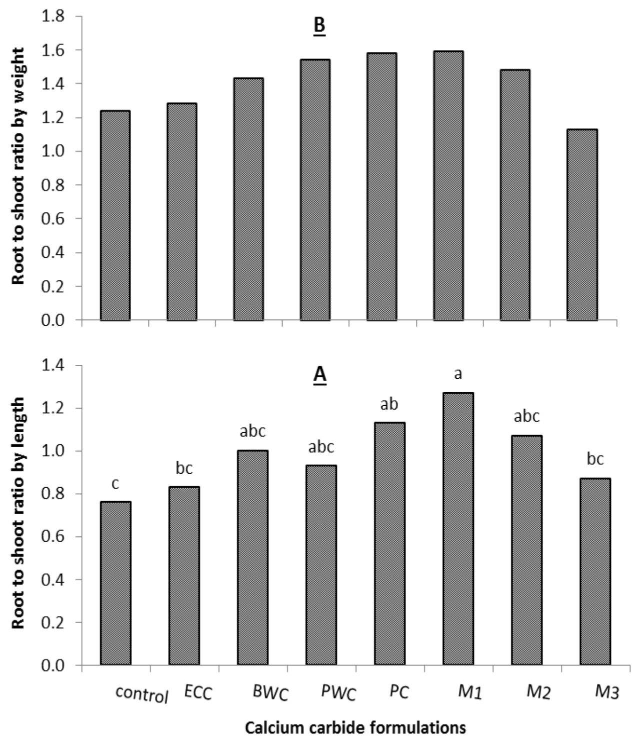
Table 3. Correlation coefficients between different parameters of wheat

	MET	ERI	TPW	RW	SW	RL	SL	R/Swt.
ERI	-0.99**							
TPW	-0.68**	0.68**						
RW	-0.67**	0.66**	0.87**					
SW	$-0.36^{N.S}$	$0.37^{N.S}$	0.69**	0.24N.S				
RL	-0.61**	0.63**	0.65**	0.72**	$0.24^{N.S}$			
SL	$-0.29^{N.S}$	$-0.31^{N.S}$	$-0.34^{N.S}$	-0.42*	$-0.06^{N.S}$	$-0.15^{N.S}$		
R/S wt.	$-0.22^{N.S}$	$0.21^{N.S}$	$0.13^{N.S}$	0.59**	-0.60**	$0.38^{N.S}$	$-0.35^{N.S}$	
R/S len.	-0.59**	0.64**	0.68**	0.75**	$0.24^{N.S}$	0.84**	-0.64**	0.44*

*, ** – significant at 0.05 and 0.01 probability level, respectively; $^{N.S}$ – non-significant

MET – mean emergence time; ERI – emergence rate index; TPW – total plant weight; RW – root weight; SW – shoot weight; RL – root length; SL – shoot length; R/S wt. – root to shoot ratio by weight; R/S len. – root to shoot ratio by length.

also reported by Abeles (1992), Sharma and Yadav (1996) and Mahmood *et al*. (2002).



ECC – Encapsulated CaC_2; BWC – Beeswax coated CaC_2; PWC – Paraffin wax coated CaC_2; PC – Paint coated CaC_2; M1 – Matrix-1; M2 – Matrix-2; M3 – Matrix-3

Figure 3. Effect of calcium carbide formulations on root to shoot ratio by length (A) and weight (B) of wheat seedlings after fifteen days of sowing

***Conclusion*:** Appropriately formulated calcium carbide can improve emergence rate index and seedling growth of wheat. Among the examined formulations, matrix-1 (21% calcium carbide, 58% polyethylene and 21% plaster of paris) at 15 mg CaC_2 kg^{-1} sand medium was found the best in improving seed germination and root:shoot ratio by length of wheat. The findings may be helpful in improving crop production of wheat in case where less and/or late seed germination, and restricted root growth are the yield limiting factors. However, the influence of formulations particularly that of matrix-1 on seed germination and seedling growth of wheat need to be further explored by considering acetylene and ethylene flux from CaC_2 incorporated soil as a parameter. Furthermore, impact of matrix-1 on seedling emergence of wheat sown in various field soils need to be investigated for making practical recommendations.

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