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RESPONSE OF OKRA (*HIBISCUS ESCULENTUS* L.) TO SOIL GIVEN ENCAPSULATED CALCIUM CARBIDE

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Abstract

Laboratory and field studies were conducted to evaluate the effectiveness of encapsulated Calcium carbide (CaC_2) as nitrification inhibitor and plant hormone. It was studied how encapsulated Calcium carbide slows down the formation of NO_3^- from applied urea by monitoring the NO_3^- and NH_4^+ contents of soil, six weeks after Calcium carbide application. Release of acetylene / ethylene was monitored from encapsulated CaC_2 after 1, 7, 15, 30 and 60 days under biotic (non-sterilization) and abiotic (complete sterilization) conditions. The effect of CaC_2 on growth and yield of okra (*Hibiscus esculentus* L.) was studied in field trial where Calcium carbide @ 0, 30 and 60 kg ha^{-1} was placed 6 cm deep in soil between the plants 2 weeks after germination. Based upon the results obtained from the laboratory and field trials, it was concluded that Calcium carbide can effectively be used as a nitrification inhibitor (acetylene) as well as plant growth regulator (ethylene). Okra showed positive response to Calcium carbide application as increase in green pod yield up to 37 % was obtained after Calcium carbide application @ 60 kg ha^{-1} of soil alongwith half of the recommended N fertilizer (60 kg N ha^{-1}) as compared to control while this increase was about 19 % compared to fertilizer alone.

Introduction

Okra (*Hibiscus esculentus* L.) commonly known as lady finger (locally called "Bhindi") is a heat loving plant of *Malvaceae* family and is one of the most important summer vegetable of Pakistan. It is also a popular home garden vegetable and a good source to fulfill the energy requirements of the body. It also provides vitamin A, B, C, protein, amino acids, minerals and iodine (Anon., 2005). The vegetable is quite palatable and liked equally by poor and rich. In Punjab, okra is sown from mid February to mid May. It brings remunerative income to growers of Punjab and Sindh Provinces. Total area under cultivation in Pakistan is 2.21×10^5 hectares and total production is about 2.86×10^6 tons of green pods (Anon., 2003-04).

In spite of all our efforts to increase okra yield in the country, it's per hectare yield is much lower than that of other agriculturally developed countries. Despite significant annual increase in fertilizer use, its yield has stagnated and even declined in some cases. So far, different conventional approaches relating to soil, plant, crop and fertilizer have been practically used to improve the yield of this vegetable. These approaches are not so successful in many cases in improving the yield or narrowing down the gap between potential and farmer's obtained yields. This situation forces us to use non-conventional approaches such as biotechnology, genetic engineering and use of plant hormones.

Ethylene is a simple, readily diffusible gaseous hormone. It regulates multiple developmental processes including seed germination, fruit ripening, abscission and senescence (Abeles *et al*., 1992). It can also stimulate elongation of certain plant organs (Kende *et al*., 1998). It prevents lodging when applied to cereals (Boutaraa, 1991).

Several studies indicate that ethylene controls and coordinates a number of growth and developmental processes, both *In vivo* and *In vitro* (Biddington, 1992; Yaseen *et al*., 2005, 2006). Being a gas, application of ethylene to plants is quite complex in practice. So efforts have been made to find out the compounds, which could release ethylene in plants or in their rhizosphere. The most important breakthrough was the development of ethephon (liquid) in 1960s, which releases C_2H_4 chemically when absorbed by the plant tissues. Since then, ethylene has been used very extensively in Europe and USA. However its use is restricted only to foliar application. Recently, after a sustained efforts of a group of co-workers at the All-Union Scientific Research Institute of Agricultural Microbiology of the Lenin and All-Union Scientific Research Institute of Agricultural Biotechnology of VASKHNIL developed a new Calcium carbide based ethylene producer "Retprol" which is used as soil amendment and breaks down slowly into acetylene and calcium upon interaction with soil water. The acetylene released is readily reducible to ethylene by soil indigenous microorganisms. The effect of this CaC_2 based formulation upon tomato and cucumber plants were investigated and yield increases upto 70 % were reported (Muromstev *et al*., 1995). Bibik *et al*., (1995) observed significant increase in number of tubers and potato yield with this soil acting ethylene producer (Retprol). In addition to hormonal action of C_2H_4 produced in the rhizosphere from the CaC_2 formulation, C_2H_2 also acts as a potent inhibitor of nitrification (Arshad & Frankenberger, 2002; Aulakh *et al*., 2001; Randall *et al*., 2001). The use of acetylene can prolong the period of existence of NH_4^+ and NO_3^- in soil which is otherwise very low due to variety of losses of these two ions on alkaline and calcareous soils of Pakistan.

The present study was carried out to investigate the effect of CaC_2 on the release of acetylene, ethylene, nitrification, growth and yield of okra crop. The use of CaC_2 based formulation could be a new and innovative approach for improving crop yield.

Materials and Methods

This study was conducted in the laboratory and experimental area of the Institute of Soil & Environmental Sciences, University of Agriculture, Faisalabad. Ethylene production in soil treated with encapsulated (Gelatin type) CaC_2 was monitored in the laboratory. Encapsulated CaC_2 @ 15 mg kg^{-1} soil was placed in the bottom of the Erlenmeyer flask (125 mL) containing 100 g soil at 60 % WHC. The soil was air-dried (2 mm $10mesh^{-1}$) and analyzed for physico-chemical properties (Table 1). The flaks were capped with mininert valves and incubated at 30 ± 2ºC. Control without CaC_2 was also performed to account for base-level C_2H_2 and C_2H_4 formation in soil. To determine production of C_2H_2 and C_2H_4, soil was sterilized by autoclaving twice at 121ºC for one hour on alternate days. CaC_2 was added (15 mg kg^{-1}) to autoclaved soil under aseptic conditions. All other conditions were the same as described for non-sterilized soil. There were three replications for all the treatments. Release of C_2H_2 and C_2H_4 was monitored after 1, 7, 15, 30 and 60 days using gas chromatography (Shimadzu-4600) fitted with a flame ionization detector (FID) and a capillary column (Porapak Q 80-100) operating isothermally under the following conditions: Carrier gas, N_2 (13 mL min^{-1}) ; H_2 flow rate, 33 $mLmin^{-1}$; air flow rate, 330 $mLmin^{-1}$; sample volume, 1mL; column temperature 70 ºC; detector temperature, 200ºC. The C_2H_2 and C_2H_4 concentrations were determined by comparison with reference standards of C_2H_2 and C_2H_4 (99.5 %) obtained from Matheson (Secancus, NJ).

Table 1. Physical and chemical properties of the experimental soil.

Property	Value
Sand (%)	48.9
Silt (%)	22.7
Clay (%)	28.4
Textural class	Sandy clay loam
Saturation (%)	34.1
pH_s	7.80
EC_e (dS m^{-1})	2.03
CEC ($cmol_c$ kg^{-1})	7.9
Organic matter (%)	0.58
$CaCO_3$ (%)	1.21
Total N (%)	0.034
NH_4^+-N (mg kg^{-1})	3.8
NO_3^--N (mg kg^{-1})	6.3

To study the effect of CaC_2 in NH_4-oxidation, 100 g soil (same soil, Table 1) in Erlenmeyer flask (125 mL) was used in the laboratory. Three levels of urea fertilizer (0, 30, 60 mg N kg^{-1} soil) were used. The fertilizer was uniformly mixed with the soil. Encapsulated CaC_2 (15 mg kg^{-1} soil) was placed at the bottom of flasks. Distilled water was used to maintain the soil moisture near field capacity (60 % WHC) up to 6 weeks from the start of experiment. Flasks were kept open at tops and incubated at 30 ± 2^oC. There were three replications for all the treatments. After six weeks, the contents of each flask were taken out and thoroughly mixed. Moist soil, equivalent to 10 g dry weight, was extracted for 1h with 100 ml of 2*M* KCl solution, containing 15 µM phenylmercuricacetate and filtered through whatman No.42 filter papers. The filtrate was analyzed for NH_4-N by the indophenol blue method and NO_3-N by a modified Griss-Ilosvay method (Kenney & Nelson, 1982).

Field experiment was conducted in 2x5 m^2 plots to verify the effect of CaC_2 on the growth and N uptake of okra. Nitrogen Fertilizer was applied @ 0, 60 and 120 kg ha^{-1} as urea. Phosphorus @ 100 kg ha^{-1} as single super phosphate and potassium @ 75 kg ha^{-1} as KCl were also applied. Half nitrogen and all phosphorus and potassium fertilizers were applied at the time of seedbed preparation while other half nitrogen was applied with Calcium carbide. Encapsulated Calcium carbide @ 0, 30 and 60 kg ha^{-1} was placed 6 cm deep in soil in between the plants two weeks after germination. Plant to plant and row to row distances were 45 and 60 cm, respectively. Green pods were collected from each plant regularly from first picking till maturity of plants. Growth parameters i.e., plant height and horizontal expansion was studied by selecting 3 plants per plot at random. Green pod yield was also recorded to investigate the response of okra to the applied Calcium carbide.

The data were analyzed statistically using completely randomized design (Steel & Torrie, 1980). Means were compared by Duncan's multiple range test (Duncan, 1955).

Results

Release of acetylene and ethylene in CaC_2 amended soil: Results indicated that there was a great difference in C_2H_2 and C_2H_4 contents of soil amended with CaC_2 under biotic and abiotic conditions (Table 2) indicating CaC_2 dependent release of C_2H_2 and its subsequent microbial transformation to C_2H_4. The gas chromatography analysis indicated that there was a large release of C_2H_2 under sterilized and non sterilized conditions i.e., 24390 nmol kg^{-1} of soil and 23100 nmol kg^{-1} of soil, respectively, which gradually decreased upto 30 days. Afterwards, there was a sharp decrease in C_2H_2 contents till 6^{th} week. This decrease in C_2H_2 contents was consistent almost with the same trend irrespective of the conditions either sterilized or not. Unlike C_2H_2, a different pattern was observed in C_2H_4 contents. After a lag period of one day C_2H_4 was observed which gradually increased to maximum (437.2 nmol kg^{-1} of soil) at the end of the incubation period (60 days). However, no C_2H_4 was detected in sterilized CaC_2 amended soil throughout the experiment, indicating that soil microbes are needed for the conversion of C_2H_2 to C_2H_4.

NH_4^+ and NO_3^- concentrations in CaC_2 amended soil: Concentrations of NH_4^+ and NO_3^- in soil amended with Calcium carbide (Table 3) indicated that under no fertilizer application, NH_4^+ and NO_3^- contents were same even if Calcium carbide was applied. Under half dose of N fertilizer application (30 mg N kg^{-1}soil), NH_4^+ contents were almost double (23.80 mg kg^{-1}soil) with CaC_2 than without it (10.21 mg kg^{-1}soil). NO_3^- contents of this treatment were also four times less with CaC_2 application (4.10 mg kg^{-1}soil) than without CaC_2 (19.60 mg kg^{-1}soil). Similarly, when full N fertilizer was applied (60 mg N kg^{-1}soil), NH_4^+ contents with CaC_2 application were four times greater (42.84 mg kg^{-1}soil) than without CaC_2 (10.60 mg kg^{-1}soil) and NO_3^- were almost 7 times higher without CaC_2 (30.10 mg kg^{-1}soil) than CaC_2 application (4.33 mg kg^{-1}soil).

Response of okra to encapsulated CaC_2: Plant height, horizontal expansion and green pod yield were significantly affected by N fertilizer alone and combination of N fertilizer plus Calcium carbide. It was observed that effect of N fertilizer on okra growth was further increased by the addition of Calcium carbide (Table 4). There was reduction in plant height by the application of N fertilizer plus Calcium carbide while increases in horizontal plant expansion and green pod yield were observed. Maximum plant height (99.5 cm) was obtained by the application of N fertilizer @ 120 kg ha^{-1}, which reduced to 90.3 cm with the addition of 60 kg CaC_2 ha^{-1}. The estimated was ~ 10 %. However, this height was 80.9 cm with half dose of N fertilizer (60 kg ha^{-1}) and 60 kg CaC_2 ha^{-1}.

Vertical reduction in plant height causes increase in horizontal expansion of plant by decreasing the internodal distance. Maximum expansion (77.7 cm) was observed where N-CaC_2, 120-60 kg ha^{-1} was applied. It is obvious from the data (Table 4) that addition of CaC_2 significantly increased the horizontal expansion of plant at all levels of N.

This increase in expansion caused increase in number of green pods per plant, which ultimately increased the total weight of green pods. Maximum green pod yield (5334 kg ha^{-1}) were obtained by the application of N-CaC_2, 60-60 kg ha^{-1}, which is ~ 37 % more than control and ~ 19 % more than fertilizer alone. Application of N_{120}-C_{60} i.e., 120 kg N ha^{-1}plus 60 kg CaC_2 ha^{-1} increased green pod yield ~ 25 % compared to control while it was ~ 8 % more than fertilizer alone.

Table 2. C_2H_2 and C_2H_4 contents in CaC_2 amended sterilized and non-sterilized soil at different intervals.

Time interval (Days)	C_2H_2 (nmol kg $^{-1}$ soil)		C_2H_4 (nmol kg $^{-1}$ soil)	
	Sterilized	Non-sterilized	Sterilized	Non-sterilized
1	24390 a	23100 a	-	19.0 d
7	20140 b	20780 b	-	115.0 c
15	19200 c	19510 c	-	156.2 bc
30	19870 c	17430 d	-	184.9 b
60	10150 d	9760 e	-	437.2 a

Values sharing same letters in a column don't differ significantly at P=0.05 according to Duncan's Multiple Range.

Table 3. Ammonium and nitrate concentrations in soil after six weeks of CaC_2 treatment at three levels of nitrogen.

N level (mg kg^{-1} soil)	NH_4^+ (mg kg^{-1}soil)		NO_3^- (mg kg^{-1})	
	-CaC_2	+CaC_2*	-CaC_2	+CaC_2
Control	3.40 d	4.75 d	4.48 c	5.22 c
30	10.21 c	23.80 b	19.60 b	4.10 c
60	10.60 c	42.84 a	30.10 a	4.33 c

Values sharing same letters in a column don't differ significantly at P=0.05 according to Duncan's Multiple Range.
*Calcium carbide was added @ 15 mg kg^{-1}.

Table 4. Response of okra to soil applied encapsulated calcium carbide.

N-C* (kg ha^{-1})	Plant height (cm)	Horizontal expansion (cm)	Yield (kg ha^{-1})	% increase over control
N_0 C_0	69.8 e	50.4 e	3889 e	-
N_0 C_{30}	67.9 e	53.8 e	4191 d	7.7
N_0 C_{60}	67.1 e	57.2 de	4338 cd	11.6
N_{60} C_0	84.9 cd	58.7 cde	4491 bc	15.4
N_{60} C_{30}	82.6 cd	64.0 bcd	4563 b	17.2
N_{60} C_{60}	80.9 d	69.3 ab	5334 a	37.0
N_{120} C_0	99.5 a	66.6 bc	4571 b	17.5
N_{120} C_{30}	95.4 ab	71.9 ab	4883 b	25.4
N_{120} C_{60}	90.3 bc	77.7 a	4152 e	6.68

Values sharing same letters in a column don't differ significantly at P=0.05 according to Duncan's Multiple Range.
*Nitrogen-calcium carbide

Discussion

Results revealed that CaC_2 acted as a potent source of C_2H_2 gas in soil which was partially reduced to plant hormone C_2H_4 (Table 2). Since no C_2H_4 was detected in CaC_2 amended sterilized (autoclaved) soil which may imply that release of C_2H_2 from CaC_2 is a chemical reaction while reduction of C_2H_2 to C_2H_4 is a strictly biotic transformation. This premise is further supported by the lag period observed in case of C_2H_4 appearance only which may imply that microorganisms were involved in reduction of C_2H_2 to C_2H_4. Similar findings have been reported by some other workers (Muromtsev *et al*., 1995; Porter, 1992; Bibik *et al*., 1995).

Application of encapsulated CaC_2 to urea amended soil resulted in slow transformation of NH_4^+ to NO_3^- (Table 3) which may imply that CaC_2 could be used as nitrification inhibitor. Calcium carbide releases copious amount of C_2H_2 which is an established inhibitor of enzyme involved in nitrification. The observed suppressive effects of CaC_2 on NH_4^+ oxidation are in conformity to the results reported by some others (Freney *et al*., 1993, 2000; Randall *et al*., 2001). Freney *et al*., (1993) reported that wax-coated CaC_2 inhibited nitrification for 60 days. It has been demonstrated in laboratory, greenhouse and field studies that coated CaC_2 provided prolonged source of C_2H_2 to minimize nitrification and decrease N losses in rice and other field crops (Crawford & Chalk 1992; Benerjee *et al*., 1990; Bronson and Mosier 1991; Chen *et al*., 1994). Bronson *et al*., (1992) and Freney *et al*., (1992) reported that CaC_2 effectively stopped nitrification in irrigated maize and wheat.

The various attributes of yield of okra were significantly affected by CaC_2 treatment. Reduction in plant height and increases in plant horizontal expansion and green pods weight of okra were observed in urea plus CaC_2 amended soil. These positive effects of CaC_2 may be due to more availability of N from the soil due to less N losses particularly as NO_3^- -N because of inhibitory effects of CaC_2 on nitrification (Yaseen *et al*., 2006). Additionally, the formation of plant hormone C_2H_4 might have also contributed in promoting the growth and yield of treated plants. Many researchers have reported C_2H_4 as a potent plant growth regulator. It has been postulated that small amounts of C_2H_4 in the rhizosphere could be physiologically active in influencing the growth and development of plants (Abeles, 1992; Muromstev *et al*., 1995; Arshad & Frankenberger, 2002). The results of laboratory studies regarding inhibitory effects of CaC_2 on nitrification and formation of C_2H_4 from C_2H_2 released from CaC_2 further support this premise.

Conclusively, it is highly likely that any formulation of CaC_2 which leads to slow and gradual release of C_2H_2/ C_2H_4 might be useful in improving the nutrient use efficiency as well as growth and yields of crops. The work on developing an appropriate formulation for coating of CaC_2 to achieve slow release of C_2H_2 as well as making it farmer friendly is in progress.

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