Proceedings:
International Symposium on
Prospects of Horticultural Industry in Pakistan
28th to 30th March, 2007
Institute of Horticultural Sciences, University of Agriculture, Faisalabad

RESPONSE OF OKRA (*Abelmoschus esculentus* L. Moench) TO SOIL APPLIED CALCIUM CARBIDE – AN INNOVATIVE APPROACH

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Abstract

Laboratory studies were conducted to investigate the release of acetylene and ethylene from encapsulated calcium carbide and how calcium carbide inhibits nitrification by studying $NH4^+$ and NO_3^- contents of soil after CaC_2 application. A pot study was also conducted where recommended dose of P and K fertilizer was used with three levels of N fertilizer i.e. 0, 60 and 120 kg ha^{-1}. CaC_2 @ 0, 30 and 60 kg ha^{-1} was applied at sowing 6 cm deep in center of the pots. Results indicated that different morphological changes were observed in okra plant after calcium carbide application. Internodal distance was reduced significantly and stem thickness was increased. Branching was observed which bear fruits as well and this characteristic was only observed when medium level of calcium carbide (30 mg kg^{-1} soil) was applied. There was more than 40% increase in green pod yield when medium level of CaC_2 alongwith fertilizer was applied compared to fertilizer alone. It was concluded that this approach can be utilized as an innovative one increase the yield of okra. Nitrification was also positively affected, thereby, increasing the fertilizer use efficiency for nitrogenous fertilizers and so decreasing the NO_3^- hazards to environment especially to ground waters.

Key words: Calcium carbide, acetylene, ethylene, nitrification inhibitor and classical triple response

INTRODUCTION

Calcium carbide (CaC_2) is a solid material that releases acetylene when applied to soil which is reduced to ethylene by soil microbes. Many researchers have conducted experiments on different crops to evaluate the response of applied calcium carbide more often as a source of "acetylene" and less as a source of plant hormone "ethylene". Acetylene is considered a potent nitrification inhibitor while ethylene is a simple, readily diffusible gaseous hormone. Ethylene regulates multiple developmental processes including seed germination, fruit ripening, abscission and senescence (Abeles et al., 1992). Exogenous ethylene application leads to morphological and physiological changes such as a higher root:shoot ratio and a decrease in stomatal conductance (Taylor and Gunderson, 1988) which could alleviate the adverse effects of drought. In most species, exogenous ethylene or ethephon stimulate the germination of dormant and non-dormant seeds (Ketring and Melouk, 1982). Studies have also shown that ethylene regulates sex expression in many monocots species including members of the Cucurbitaceous family (Rudich, 1990). It effectively reduces plant height and lodging when applied to cereal crops (Boutaraa, 1991). Seedlings also exhibit a Classical Triple Response to higher doses of ethylene consisting of shortened, thickened hypocotyls and a pronounced apical hook (Abeles et al., 1992).

Being a gas, application of ethylene to field grown plants is quite complex in practice. So efforts have been made to develop the compounds, which could release ethylene in plants or in their rhizosphere. The most important break through was the development of ethephon (liquid) in 1960s, which releases C_2H_4 chemically when absorbed by the plant tissues (Abeles et al., 1992; Arshad and Frankenberger, 2002). However, its use is restricted only to foliar application. Recently, after a long joint effort of a group of co-workers at the All-Union Scientific Research Institute of Agricultural Microbiology of the Lenin resulted in a new CaC_2 based C_2H_4-producing formulation under the trade name of "Retprol" which is used as soil amendment and breaks down slowly into acetylene and calcium upon interaction with soil water. The acetylene released is reducible to ethylene by soil indigenous microorganisms. Calcium carbide being a solid material can be used as a promising source of acetylene (Aulakh et al., 2001). Acetylene is an effective inhibitor of nitrification and de-nitrification (Bleecker and Kende, 2000; Thompson, 1996). This gas restricts the activity of the enzyme involved in the ammonia-oxidizing process. Since nitrification ($NH_4^+ \rightarrow NO_3^-$) ultimately leads to the loss of N either by leaching as NO_3^- or denitrification and volatile oxides of N to the atmosphere. Retaining of N in the ammonical (NH_4^+) form in soil over an extended period may decrease the NO_3^- losses to ground water or losses in the form of nitrogen oxides to the atmosphere (Freney et al., 2000). Thus reduced rates of nitrification in soil may result in increased N fertilizer use efficiency (Keerthisinghe et al., 1996). To extend the period of acetylene/ethylene in soil, CaC_2 can be used with some coating to release these gases slowly (Banerjee and Mosier, 1989; Keerthisinghe et al., 1993). In an experiment a soil acting ethylene producer based on calcium carbide (Retprol) increased number and yield of potato seed tubers 30-54% in cv. Luk'yanovskii and 61-121% in cv. Lugovskoi (Bibik et al., 1995). CaC_2-based ethylene- producing preparation was a more cost effective and environmental friendly than synthetic sources of ethylene (Muromstev et al., 1995). Muromstev et al., (1993) already suggested using the formulated CaC_2 to increase the concentration of C_2H_4 in the rhizosphere of plant. An improvement in yield of wheat, cotton, rice and maize crops in response to application of coated CaC_2 which released C_2H_2 slowly had already been reported (Keerthisinghe et al., 1996; Randall et al., 2001).

Okra (*Abelmoschus esculentus*) 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 not only in Pakistan but throughout the world. It is popular home garden vegetables and a good source to fulfill the energy requirements of the body. No work has been conducted on okra (*Abelmoschus esculentus*) in the world to investigate the response of crop to calcium carbide. Therefore, this study was conducted to investigate its response to calcium carbide as a non conventional approach for increasing yield of this vegetable.

MATERIALS AND METHOD

This study was conducted in the laboratory and experimental area of the Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad, Pakistan. Ethylene production in soil treated with encapsulated CaC_2 was monitored in the laboratory. Encapsulated CaC_2 @ 30 mg kg^{-1}soil was placed in the bottom of the Erlenmeyer flask (125 ml) containing 100 g soil at 60% WHC. 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**.**

The effect of CaC_2 as an inhibitor of nitrification was also studied in the laboratory. Encapsulated CaC_2 @ 30 mg kg^{-1} soil was placed 6 cm deep in the center of each beaker containing 100 g soil. After six weeks, the contents of each beaker were taken out and thoroughly mixed. Moist soil, equivalent to 10 g dry weight, was extracted for one hour 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^+ and NO_3^- according to the methods described by Keeney and Nelson (1982).

To study the Classical Triple Response of okra to ethylene, pot and field experiments were conducted. For pot experiment 12.5 kg soil was filled in earthen pots lined with polyethylene bags. This experiment was mainly to study morphological changes in plant and pod. Both the experiments were conducted with similar set of treatments. N Fertilizer was applied @ 0, 60 and 120 kg ha^{-1}. Encapsulated calcium carbide @ 0, 30 and 60 mg kg^{-1} soil was placed 6 cm deep in soil (root zone) and in the center of pot two weeks after germination. Nitrogen as urea while phosphorus (90 kg P_2O_5 ha^{-1}) as single super phosphate and potassium (60 kg K_2O ha^{-1}) as sulfate of potash. Half of N and whole phosphorus and potassium were applied at sowing time while other half nitrogen after two weeks of germination. Green pods were collected from each plant regularly from first picking till maturity of plants and their fresh weight was recorded. Growth parameters for plant height, stem girth, plant horizontal expansion, internodal distance, root dry matter and pod shape was studied. Root, stem and pods were dried in an oven at 70 ˚C and ground. Then 0.5 g of each plant part was digested to determine nitrogen concentration by the method of Hu and Barker (1999). Data were analyzed statistically according to the methods described by Steel and Torrie (1980).

RESULTS AND DISCUSSION

Acetylene/ethylene contents of soil after CaC_2 application

Results indicated that there was a great difference between C_2H_2 and C_2H_4 contents of soil under biotic and abiotic conditions (Table 1). Almost same pattern was observed for C_2H_2 under sterilized and non sterilized conditions. C_2H_4 studies indicated that from first day up to sixty days under sterilized conditions, no acetylene was converted to ethylene where as under non-sterilized conditions after seven days acetylene conversion to ethylene (15 nmol kg^{-1} of soil) was recorded which increased with time (137.2 nmol kg^{-1} of soil after 60 days). This indicated that soil microbes are needed for the conversion of C_2H_2 to C_2H_4 as no conversion was observed under sterilized conditions. Similar results reported by Khalid et al. (2006) and Yaseen et al. (2006)

Table 1: C_2H_2 and C_2H_4 contents of CaC_2 amended sterilized and non-sterilized soil as observed after different intervals (average of three replications).

Time interval (Days)	C_2H_2 (nmol kg^{-1} soil)		C_2H_4 (nmol kg^{-1} soil)	
	Sterilized	Non Sterilized	Sterilized	Non sterilized
1	24390a	23100a	-	-
7	20140b	20780b	-	15.0d
15	19200b	19510bc	-	56.2c
30	19870b	17430c	-	84.9b
60	10150c	9760d	-	137.2a

Values in columns having different letter (s) differ significantly at $P \leq 0.05$

Calcium carbide as nitrification inhibitor

NH_4^+ and NO_3^- contents of soil under laboratory conditions were studied after application of calcium carbide. Results indicated that under no fertilizer application, NH_4^+ and NO_3^- contents were almost same even if calcium carbide was applied (Table 2). When calcium carbide was applied with fertilizer, NH_4^+-N was two to four times with than without CaC_2. On the other hand NO_3^--N was almost 5-7 times higher without CaC_2 as compare to fertilizer CaC_2 application. It is quite well-known now that presence of both NH_4^+ and NO_3^- is more beneficial as a source of plant N rather than the either available alone; CaC_2 may help to create this situation. One of the products of CaC_2 decomposition i.e., acetylene, is inhibitory to nitrification. Therefore, a part of the fertilizer N will remain in NH_4- form over extended periods of time resulting in the plant availability of both NO_3^- and NH_4^+. Similar results were observed by Banerjee and Moiser (1989), Smith et al. (1993), Freney et al. (2000), Chen et al. (2000) and Yaseen et al. (2005, 2006).

Table 2: NH_4^+ and $NO3^-$ contents of soil after six weeks with and without CaC_2 at three N fertilizer levels

N level (mg kg^{-1} soil)	NH_4^+ (mg kg^{-1})		NO_3^- (mg kg^{-1})	
	Without CaC_2	With CaC_2	Without CaC_2	With CaC_2
0 (control)	3.40b	4.75c	4.58c	5.22a
60	10.21a	23.80b	19.60b	4.10b
120	10.60a	42.84a	30.10a	4.33ab

Values in columns having different letter(s) differ significantly at $P \leq 0.05$

Influence of calcium carbide on germination of seeds

Okra seed were soaked in water for 8 hours prior to sowing (seed priming). After 4 days of sowing in pots data was collected regarding seed germination. Results indicated that there was rapid seed germination after calcium carbide application (Figure 1). Under control (without fertilizer) root development was just initiated, with fertilizer applications thread like primary root was observed whereas when calcium carbide was applied with fertilizer, well developed primary root with thread like secondary roots was observed. This development of root system may be attributed to the calcium ions released when CaC_2 reacts with water to produce acetylene.

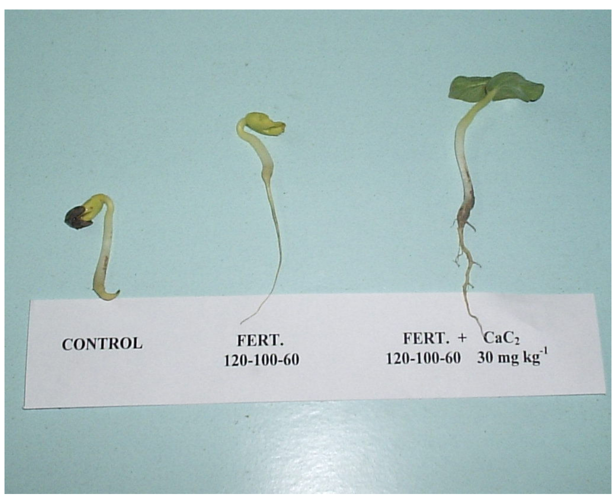

Figure 1: Root after 4 days of sowing in pots

Classical triple response of okra to calcium carbide

Results indicated that okra plant showed a Classical Triple Response to CaC_2 application i.e. reduction in plant height (Table 3), hook formation of green pod (Figure 2) and reduction in internodal distance (Figure 3 & Table 3). Plant height was almost unaffected under no N fertilizer application even with the application of alone calcium carbide @ 30 and 60 kg ha^{-1}. At the highest N level, plant height was significantly reduced with addition of calcium carbide compared to alone N fertilizer. Similarly plant horizontal expansion and stem girth were also increased with CaC_2 application (Table 3). It is also clear from the data that reduction in plant height was mainly due to reduction in internodal distance which was also due the addition of CaC_2 (Figure 3).

Figure 2: Hook formation of pod after CaC_2 application

Figure 3: Inter-nodal distance with and without CaC_2

Table 3: Classical triple response of okra to applied calcium carbide (average of three replications)

Treatment	Plant Height (cm)	Horizontal expansion (cm)	Inter-nodal distance (cm)
$N_0 C_0$	69.7f	40.2 g	5.7b
$N_0 C_{30}$	67.9fg	43.5f	5.6b
$N_0 C_{60}$	67.1 g	45.6ef	5.7b
$N_{60} C_0$	87.9cd	46.8e	5.9ab
$N_{60} C_{30}$	82.6d	51.2d	5.3c
$N_{60} C_{60}$	77.3e	55.4bc	4.7d
$N_{120} C_0$	99.2a	53.3c	6.2a
$N_{120} C_{30}$	95.4b	57.4b	5.0cd
$N_{120} C_{60}$	90.3c	62.3a	4.51e

N_0, N_{60} & N_{120} means N @ 0, 60 and 120 kg ha^{-1} soil, respectively.
C_0, C_{30} & C_{60} means Calcium carbide @ 0, 30 and 60 kg ha^{-1} of soil, respectively
Values in columns having different letter (s) differ significantly at $P \leq 0.05$

Effect of application of calcium carbide on plant morphology

Application of calcium carbide @ 30 and 60 kg ha^{-1} in the presence of N fertilizer increased growth and green pod yield. At 30 mg kg^{-1} (medium level), branching in okra plant was observed (Figure 4). These branches also beard pods which contributed to increase green pods yield. Branching is not an ordinary character of this plant which might be due to the application of calcium carbide, however it was not observed at all levels of calcium carbide except the medium one. At higher levels (60 kg ha^{-1}) plant was adversely affected and morphological disorders were observed as are shown in Figure 5.

Effect of calcium carbide application on nitrogen concentration and green pod yield

There was a positive effect of calcium carbide on N contents of shoot, root and pod. This indicated that application of calcium carbide in the presence of N fertilizer improved the recovery of applied N fertilizer thereby decreasing the losses. There was no pronounced difference in green pod yield with alone CaC_2 application. A green pod yield of 7.63 t ha^{-1} was recorded with N_{60} C_{60} against 5.34 t ha^{-1} with alone N fertilizer (60 kg N ha^{-1}). The estimated increase in green pod yield was more than 40% (Table 4). Increased yield of okra with the application of CaC_2 may be due to enhanced uptake of nutrients by plant due to nitrification inhibitory effect of acetylene or production of ethylene from CaC_2 which induced increase in root primordia which increases the explored volume of soil for nutrient acquisition. In addition to increased availability of nutrients, CaC_2 may also lead to changes in the form of available N. It is quite well-known that presence of both NH_4^+ and NO_3^- is more beneficial as a source of plant N rather than the either available alone. Nitrification inhibitors have indeed been reported to improve crop yields not only by maintaining higher levels of NH_4^+ but by decreasing the losses of N through denitrification and NO_3^- leaching as well. In addition, CaC_2 may also serve as a supplemental source of Ca which is useful for plant growth. Enhanced availability of Ca has also been reported to improve the uptake and assimilation of NO_3^- thereby suggesting additional utility of CaC_2. Yaseen et al. (2006) reported similar results in agronomic crops.

Figure 4: Branching of plant after CaC_2 application

Figure 5: Morphological disorders observed after CaC_2 application

Table 4: Effect of calcium carbide on N concentration and green pod yield of okra (average of three replications)

Treatment	Shoot N (%)	Root N (%)	Pod N (%)	Yield (t. ha^{-1})
$N_0 C_0$	0.51e	2.09c	1.91c	3.89d
$N_0 C_{30}$	0.65d	1.98c	1.97c	4.19cd
$N_0 C_{60}$	0.75c	1.82c	2.08c	4.49c
$N_{60} C_0$	0.78c	2.59b	2.27b	5.34b
$N_{60} C_{30}$	0.85b	3.42a	2.69ab	6.23ab
$N_{60} C_{60}$	0.85b	3.59a	3.02a	7.63a
$N_{120} C_0$	0.83b	3.47a	2.79ab	5.88b
$N_{120} C_{30}$	0.93ab	3.59a	2.91a	6.29ab
$N_{120} C_{60}$	1.02a	3.62a	3.02a	4.55c

N_0, N_{60} & N_{120} means N @ 0, 60 and 120 kg ha^{-1} soil, respectively.
C_0, C_{30} & C_{60} means Calcium carbide @ 0, 30 and 60 kg ha^{-1} of soil, respectively
Values in columns having different letter (s) differ significantly at $P \leq 0.05$

CONCLUSION

Calcium carbide can be effectively used as a potent source of gases that inhibit nitrification on one hand and on the other promote growth and yield either due to improved nitrogen use efficiency or stimulatory effect of plant hormone "ethylene".

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