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## SUMMARY

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The use of plant growth regulators (PGRs) is becoming an important element in the modern technology of agricultural production. Ethylene is a potent PGR of the most practical value as it performs diverse physiological functions in plants. In some cases, the presence of ethylene is stimulatory while in others it is inhibitory depending upon its concentration and sensitivity of the exposed tissue.

Being a gas, application of ethylene for the treatment of vegetative 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 breakthrough was the development of ethephon (a liquid source of ethylene) in 1960's which releases  $C_2H_4$  chemically when absorbed by the plant tissue. Since then, ethylene has been used extensively in Europe, USA and other countries. However, its use was restricted only to foliar application.

Although, it was established long ago that soil microbiota are the potential producers of  $C_2H_4$ , however, after a long joint efforts of a group of co-workers at the All-Union Scientific Research Institute of Agricultural Microbiology of the Lenin All-Union Academy of Agricultural Science (VASKHNIL), the All-Union Scientific Research institute of Agricultural Biotechnology of VASKHNIL, and the Ukrainian Scientific Research Institute of Irrigation Farming, developed a new ethylene producer in soil under the name of "Retprol". Retprol is a calcium carbide ( $CaC_2$ ) based formulation, which is used as soil amendment and breaks down slowly into acetylene and calcium hydroxide upon interaction with soil water. The acetylene released is readily reducible to ethylene by soil indigenous microorganisms. The effects of this  $CaC_2$  based formulation were investigated on tomato and cucumber plants and yield increases

up to 70 % were reported. Significant increase in number of tubers and potato yield with this soil-acting ethylene producer was also observed by another group of workers.

Yields of vegetables per unit area are relatively low. Vegetables like okra, tomato and chilies etc are the leading vegetable crops of Pakistan. Being the diet of vast majority of people, these occupy central position in agricultural policies. There is need to increase the production of these vegetables substantially by using non-conventional approaches. The use of  $\text{CaC}_2$  based formulation could be a new and innovative approach to develop a cost effective formulation for enhancing vegetable yields. Keeping this in view, present project is planned to assess and evaluate potential of  $\text{CaC}_2$  to improve green pod yield in okra by increasing fertilizer use efficiency. Besides this, information generated from this project could be used for preparation of technology transfer to the farmers.

Keeping this in view, present project was planned to investigate the effect of  $\text{CaC}_2$  on release of acetylene, ethylene, nitrification, seed germination, growth and yield of okra crop as well as molecular changes especially protein and DNA.

A series of experiments were conducted to evaluate encapsulated calcium carbide (ECC) as (a) potent source of acetylene and ethylene gases in soil (b) their role as a nitrification inhibitor (acetylene) to improve nitrogen economy of soil and plant hormone (ethylene) to stimulate plant growth and (c) effect of ECC on growth and yield of okra (*Hibiscus esculentus* L.). Laboratory experiment was conducted to monitor the release of acetylene and its conversion into ethylene over intervals in sterile and non-sterile soil conditions over a period of 60 days from applied ECC. Pot experiment was conducted to evaluate the role of ECC as a nitrification inhibitor by studying  $\text{NH}_4^+$  and  $\text{NO}_3^-$  contents in the soil after ECC application with and without urea fertilizer. Another pot experiment was conducted where recommended dose of P and K fertilizers were used with three levels of

nitrogen fertilizer (0, 30 and 60 mg N kg<sup>-1</sup> soil) as urea. Encapsulated calcium carbide @ 0, 15 and 30 mg kg<sup>-1</sup> was applied two weeks after germination 6 cm deep in the center of pots. A field experiment was conducted to verify the results of the above experiments. The results of experiments further verified by studying the changes in proteins and DNA contents in the plant treated with calcium carbide.

This study demonstrated the effectiveness of encapsulated CaC<sub>2</sub> under N fertilized conditions for improving the growth and yield of okra by acting as a source of C<sub>2</sub>H<sub>2</sub> (nitrification inhibitor) and C<sub>2</sub>H<sub>4</sub> (plant hormone). Laboratory studies revealed that CaC<sub>2</sub> acted as a potential source of C<sub>2</sub>H<sub>2</sub> in soil, which was partially reduced to C<sub>2</sub>H<sub>4</sub> over a period of time. Since no C<sub>2</sub>H<sub>4</sub> was detected in CaC<sub>2</sub>-amended sterilized soil which may imply that the release of C<sub>2</sub>H<sub>2</sub> from CaC<sub>2</sub> is a chemical reaction while reduction of C<sub>2</sub>H<sub>2</sub> to C<sub>2</sub>H<sub>4</sub> is a biochemical transformation. This premise is further supported by a lag period observed only in the case of C<sub>2</sub>H<sub>4</sub> formation. This implies that microorganisms are involved in the reduction of C<sub>2</sub>H<sub>2</sub> to C<sub>2</sub>H<sub>4</sub>. Other scientists have reported involvement of microorganisms in the synthesis of C<sub>2</sub>H<sub>4</sub> in soil and in pure cultures (Muromtsev *et al.*, 1991; Bibik *et al.*, 1995; Akhtar *et al.*, 2005).

Application of encapsulated CaC<sub>2</sub> significantly suppressed the oxidation of NH<sub>4</sub><sup>+</sup> to NO<sub>3</sub><sup>-</sup> in a soil-amended with N fertilizer (urea). This suggests that being a source of C<sub>2</sub>H<sub>4</sub>, CaC<sub>2</sub> could be used as a nitrification inhibitor. The observed suppressive effect of CaC<sub>2</sub> on NH<sub>4</sub><sup>+</sup> oxidation is also supported by the findings of Freney *et al.* (1992, 2000) and Randall *et al.* (2001). Similarly, scientists have suggested that coated CaC<sub>2</sub> can provide a prolonged source of C<sub>2</sub>H<sub>2</sub> to minimize nitrification and decrease N losses in rice and other field crops (Banerjee *et al.*, 1990; Bronson and Mosier, 1991; Crawford and Chalk, 1992; Chen *et al.*, 1994).

When  $\text{CaC}_2$  was applied alone and compared with control, it was concluded that maximum concentration of ethylene was produced in 30 mg  $\text{CaC}_2$  treatment whereas at high level of 45 mg  $\text{CaC}_2$ , no seed germination was observed. There was an appearance of callus on seeds in 25 mg and 30 mg  $\text{CaC}_2$  level that most probably might contribute to early development of secondary roots in okra plant.  $\text{CaC}_2$  application with STS showed maximum ethylene production by seeds receiving 45 mg  $\text{CaC}_2$  with 50  $\mu\text{M}$  STS. There was also a delay in germination in 15 mg  $\text{CaC}_2$  level with STS most probably this level of  $\text{CaC}_2$  was not able to produce acetylene/ethylene required for the compensation of the inhibitory effect of STS but at higher levels of  $\text{CaC}_2$ , this inhibitory effect of STS was compensated and seeds were germinated. More over at 30 and 45 mg  $\text{CaC}_2$  with STS, callus was also present. Considering  $\text{CaC}_2$  with AVG application, maximum ethylene was observed at highest  $\text{CaC}_2$  level (45 mg  $\text{CaC}_2$ ) but there was germination of seeds at all the levels and the roots were with callus, indicating that AVG was not able to block the action of that part contributed by  $\text{CaC}_2$  needed for callus formation. When  $\text{CoCl}_2$  was applied with  $\text{CaC}_2$ , again there was highest ethylene at 45 mg  $\text{CaC}_2$  level coupled with 50  $\mu\text{M}$   $\text{CoCl}_2$ . It was observed that application of  $\text{CaC}_2$  with  $\text{CoCl}_2$  delayed germination. Regarding the individual effects of ethylene biosynthesis / action inhibitors, almost equal amount of ethylene was observed in control, 50  $\mu\text{M}$  STS and 0.5 mM AVG but with  $\text{CoCl}_2$  almost half ethylene was produced by seed during germination. There was no germination of seed when alone STS and  $\text{CoCl}_2$  were applied except control and AVG treatments.

Results also revealed that encapsulated  $\text{CaC}_2$  at the rate of either 30 mg  $\text{kg}^{-1}$  plus half of N fertilizer or 15 mg  $\text{kg}^{-1}$  plus full dose of N fertilizer had significant stimulatory effect on the growth and yield of okra. These positive effects of  $\text{CaC}_2$  in the presence of N fertilizer could be attributed to the physiologically active concentration of plant hormone  $\text{C}_2\text{H}_4$  as well as higher

availability of N in the rhizosphere. A gradual formation of physiologically active concentration of  $C_2H_4$  from the microbial reduction of  $C_2H_2$  might have also contributed in root growth promotion, which subsequently resulted in better shoot growth and yield of treated plants due to reduction in internodal distance and increase in plant horizontal expansion to increase the number of flower buds. Researchers have reported  $C_2H_4$  as a potent plant growth regulator (Abeles, 1992; Muromstev *et al.*, 1991; Arshad and Frankenberger, 2002). It has been postulated that a very small amount of  $C_2H_4$  in the rhizosphere could be physiologically active in influencing the growth and development of plants. Additionally, reduced  $NH_4^+$ -oxidation due to  $C_2H_2$  might also have resulted in a higher uptake of  $NH_4^+$ -N, saving energy required by the plant to assimilate  $NO_3^-$  into metabolic process for the development of a plant. This premise is supported by the fact that the recovery of applied N by plants was enhanced as a result of combined application of encapsulated  $CaC_2$  and N fertilizer compared with the application of N fertilizer alone. Many researchers have reported that  $CaC_2$  acts as nitrification inhibitor in soil (Banerjee *et al.*, 1990; Aulakh *et al.*, 2001).

The results also indicated that application of  $CaC_2$  2 weeks after sowing of okra seed gave promising effect as ethylene released from  $CaC_2$  could stimulate root growth better at early growth stages, consequently improve shoot growth. Scientists have reported that  $C_2H_4$  stimulates root growth of seedlings (Sharma and Yadav, 1996; Freney *et al.*, 2000).

As expected, encapsulated  $CaC_2$  application in urea amended soil significantly improved the N uptake in okra that resulted into higher N-use efficiency of applied N fertilizer. This may mainly be due to suppressing effect of  $C_2H_2$  gas released from  $CaC_2$  on the oxidation of  $NH_4^+$  to  $NO_3^-$ . Moreover, better root growth in response to  $C_2H_4$  might have also contributed in greater uptake of nutrients including N.

Results of the experiment also indicated increase in protein contents and reduction in DNA contents in stem part of the plant. Calcium carbide application also induced molecular changes i.e. changes in protein types at different molecular weight (kDa) in root, shoot, leaf and fruit of okra.

Finally, it may be concluded from the results of experiments that calcium carbide proved a better source of acetylene and ethylene which on one side gave better N fertilizer use efficiency there by reducing  $\text{NO}_3^-$  hazards in ground water and on the other hand good solid source of ethylene for plants to increasing yield of green pods in okra. Application of calcium carbide in combination with N fertilizer increased green pod yield from 27 to 37 % over alone fertilizer application in different experiments. Therefore, it can be used as a non conventional approach to increase okra yields in the country.