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Residue analysis and bulb sprouting in CIPC treated onion

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ABSTRACT

The effect of CIPC (isopropyl N-(3-chlorophenyl) carbamate), commonly known as chlorpropham was studied on different onion varieties as preharvest application in *rabi* and *kharif* seasons, as well as postharvest application on *rabi* produce. Three varieties, *viz*. Bhima Kiran, B. Shakti and N-2-4-1 were used in *rabi* season for pre- and post-harvest applications (hot fogging) in *kharif* season on B. Raj and B. Red. Sprouting of bulbs during storage was not affected when CIPC was applied as preharvest or postharvest treatment in *rabi* crop. However, the sprouting was significantly reduced when CIPC was sprayed as a preharvest application in *kharif* season. The spraying of 2% CIPC 75 days after planting was found to be the best treatment. The residues of CIPC in the *rabi* season (preharvest application) were as low as 0.08 mg/kg to a maximum of 1.84 mg/kg fresh weight. In the postharvest application, the CIPC residue ranged between 0.37 and 0.61 mg/kg fresh weight and residues were always below the permissible levels. The CIPC was found useful for forced ripening of *kharif* crop as phyto-toxicity symptoms were apparent in the studies conducted in *kharif* season when CIPC concentration was 1% or above.

Key words: CIPC, hot fogging, onion, phyto-toxicity, preharvest application, postharvest application.

INTRODUCTION

The physiological weight loss, microbial decay and sprouting are the three major constraints in onion storage (Murkute and Gopal, 12). Albeit, the physiological weight loss and microbial decay may be reduced significantly by the use of cold storage and clean postharvest management practices (Benkeblia, 2; Lawande and Murkute, 7). However, sprouting, *i.e.*, the resumption of growth, which eventually protrudes from the bulb, is still a main impediment for long term storage. Extended suppression of sprout growth in onion was achieved using maleic hydrazide (MH), a synthetic sprout suppressant. However, carcinogenic activity of MH (Epstein *et al*., 6) does not allow its practical use. Thus, the identification and standardization of alternative phytohormones/ agrochemicals for pre-harvest treatments in onion is important.

Among chemical treatments, isopropyl N-(3-chlorophenyl) carbamate commonly known as CIPC or chlorpropham, has been found to have sprout suppressant activity and is being widely used in potato (Mahajan *et al*., 8). Spray application of CIPC at the rate of 40 and 60 ml/ tonne of potatoes significantly reduced sprouting, suppressed sprout growth and reduced total storage losses up to 90 days under heap (18-32°C, 52-88% RH) and pit (19-27°C, 69-92% RH) storage. CIPC treatment was also not found to affect the quality of stored potatoes and therefore is being adapted at commercial scale in potatoes (Mehta *et al*., 10). In India, the *rabi* crop, which is often stored for about six months, to meet domestic requirements has losses due to sprouting up 10-12%. (Lawande and Murkute, 7; Murkute, 11). Further, *kharif* crop, which is needed to stabilize the market prices, is highly prone to sprouting (Murkute and Gopal, 12).

The carbamate isopropyl N-phenyl (CIP), of which CIPC is a chlorinated form, significantly reduced sprouting in onion when applied as a postharvest application (Benkeblia, 2). However, there is acute dearth of information on the efficacy of CIPC to restrict sprouting and its residues in onion *vis-á-vis* phytotoxicity if applied on standing crop. Further, the methodology of postharvest application of CIPC through fogging needs to be studied as dipping of bulbs in the chemical solutions will affect the curing of onion. The present study was contemplated to find whether CIPC can be used for forced ripening in onion and record the physical tolerance limit of CIPC for pre-harvest application *vis-á-vis* its residue levels if applied both as a pre- and also as postharvest treatments.

MATERIALS AND METHODS

In all experiments, the source of CIPC (50% H.N.) used was from United Phosphorus Ltd., Mumbai, India. The field experiment was laid out in *rabi* season (in 2010-11) in randomised block design (RBD) with three replications having a gross plot size of 5.40 m x 4.40 m^2. The experiment was carried out in for a pre-harvest application on three varieties, *viz*.,

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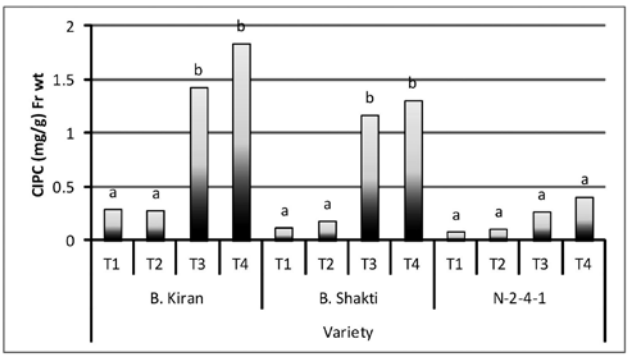
Bhima Kiran, B. Shakti and N-2-4-1. The CIPC was sprayed at 0.05 and 0.5% after 90 and 105 DAP. The second experiment was carried out as a postharvest application on *rabi* harvest (May 2011) of the above varieties. CIPC hot fogging was done (@ 50 g active ingredient (a.i.) /tonne) on onion bulbs. The third experiment was carried out in *kharif* season (in 2011) again as a preharvest application on two varieties, *viz*., B. Raj and B. Red with three replications in RBD having a gross plot size of 6.0 m x 1 m. The CIPC was sprayed in 1.0, 1.5 and 2.0% after 60, 75 and 90 DAP. All other cultivation and postharvest practices were followed as per the standard recommendations for the onion crop.

Isopropyl N-(3-chlorophenyl) carbamate (CIPC) residues were estimated by the method developed and standardized on high performance liquid chromatograph (HPLC) by Singh and Ezekiel (15). A composite sample from five onion bulbs was used for the analysis of residues. The extraction was done using n-hexane and 10 g each of anhydrous sodium sulfate (E-Merck India Ltd., Mumbai) and Kieselguhr (HiMedia Lab Ltd., Mumbai) were added in the mixture. The extract was filtered and reduced to near dryness in 30 ml glass vials below 30ºC to avoid CIPC loss due to volatilization. Water used in the mobile phase was Milli-Q grade (Millipore Inc., USA) having resistance of more than 14 ohms. Standard (CIPC) used was obtained from Sigma Chemical Co. (USA). The residues were analyzed using the following conditions: Injector: Rheodyne injector with starter switch, pump: Lachrom L-7100 isocratic (Merck-Hitachi, Dermstadt, Germany), column: 125 x 4 mm Purospher RP-18e column, oven: Lachrom L-7350 (Merck-Hitachi), detector: Lachrom L-7420 UV-visible detector (Merck-Hitachi), mobile phase: Methanol: acetonitrile: water in 35:35:30 ratio, flow rate: 1 ml min^{-1}, column temperature: 35ºC, injection volume: 20 µl, detector wavelength and absorbance: 236 nm set at 0.04 AUFS, retention time: 3 min. from injection. The concentrated extract was dissolved in 200 µl of HPLC grade methanol and 20 µl of this sample (equivalent to 1 g fresh bulb tissue) was injected into the system for quantification. The residues in bulb samples were quantified by comparing the peak area with that of standard curve.

The emergence of leaf primordial up to 0.5 cm out of neck was regarded as sprouting of bulb in all experiments. About 200-220 onion bulbs were taken in three replicates to record the data on sprouting. The data on sprouting in *rabi* season were recorded after every two months up to six months, while in *kharif,* monthly observations were recorded up to three months and expressed in percentages. The storage experiment was conducted in completely randomized design (CRD).

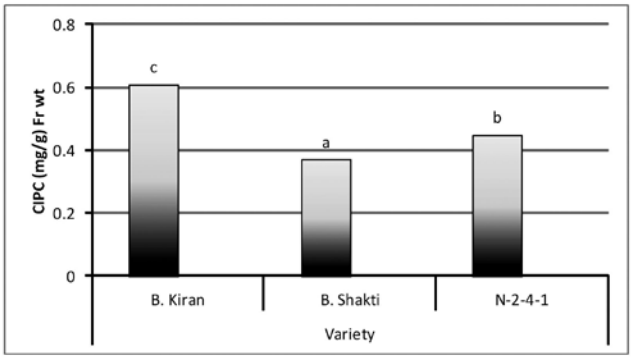
RESULTS AND DISCUSSION

The preharvest application of CIPC in the *rabi* season resulted in as low as 0.08 mg/ kg fresh weight to a maximum concentration of 1.84 mg/ kg fresh weight (Fig. 1). Higher concentrations of residues were recorded in the treatments receiving higher concentration of CIPC. Residues were absent in control plots. However, irrespective of the concentration, the treatment done at 105 DAP showed higher CIPC concentration than the treatment at 90 DAP. In the 105 DAP treatment, the residues were significantly higher in B. Kiran and B. Shakti than N-2-4-1 for all the concentrations of CIPC. Phytotoxicity symptoms were not observed in any of the treatments during *rabi* season. In the postharvest application on the *rabi* season produce, the CIPC residues ranged from 0.37 to 0.61 mg/ kg fresh weight (Fig. 2). The genotypic differences in residues were significant and



T1: CIPC @ 0.05% spray at 90 DAP; T2: CIPC @ 0.5% spray at 90 DAP; T3: CIPC @ 0.05% spray at 105 DAP; T4: CIPC @ 0.5% spray at 105 DAP
Different lowercase letters on the top of columns represent significant difference at $p \leq 0.05$.

Fig. 1. CIPC residues in different onion varieties after preharvest application in *rabi* season.



Different lowercase letters on the top of columns represent significant difference at $p \leq 0.05$.

Fig. 2. CIPC residues in different onion varieties after postharvest application on *rabi* season produce.

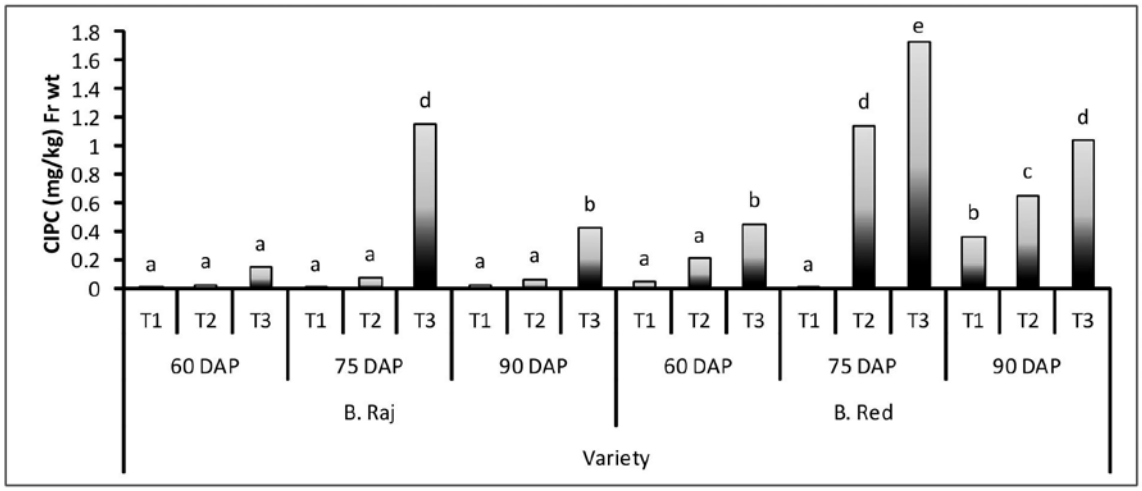
in B. Kiran the concentration was the highest followed by N-2-4-1 and B. Shakti, respectively.

The preharvest application in *kharif* season showed that the residues ranged from as low as 0.01 mg/kg fresh weight to a highest concentration of 1.74 mg/ kg fresh weight (Fig. 3). The residues were higher in bulbs treated with 2% concentration at 75 DAP as compared to lower concentrations in all the varieties. The differences in residues due to date of treatment were also observed being particularly higher in concentrations in 75 and 90 days. The level of residues decreased at 90 DAP as compared to application 75 DAP. Residues were absent in control plots. Phytotoxicity symptoms were prominent in all treatments as the foliage dried up fully after two days of the scheduled spraying of CIPC. However, the resumption of new growth was observed after 10-12 days, when left un-harvested.

The residues observed in the samples in all experiments were within the recommended minimum residue level (MRL) of 30 mg/ kg as given by Environmental Protection Agency (EPA, 5) and 10 mg/ kg as recommended by European Union (Anon, 1) for potatoes. In general, abscisic acid (ABA) is believed to be associated with the dormancy of bulbs in onion and results have revealed that exogenous treatment of ABA (10 µM) could restrict the sprouting to 2.9% after 100 days (Chen *et al*., 4). However, Campbell *et al*. (3) demonstrated that sprout repression by CIPC is not a function of elevated ABA in potato. Seetharaman and Mondy (14) revealed that propham (CIP) or its chlorinated form CIPC prevents the translocation of nitrogenous compounds towards the sprout and the retention of nutrients in the reserve tissues resulting in the sprout suppression.

The residues recorded in the treated samples in all experiments were very low as compared to the concentration of CIPC used for spraying in preharvest applications. The highest concentration of 1.74 mg/kg fresh weight was recorded in postharvest applications, which is very low as compared to potatoes. Single spray application of a commercial formulation of CIPC @ 20 mg a.i. /kg potatoes has been reported to give residues of 0.81-3.85 mg/ kg fresh tuber weight in peels. The highest concentration of CIPC reported in potato peels was 20.17 mg/kg fresh weight, whereas in unpeeled and peeled tubers the residue levels were reported to be very low ranging from 0.29 to 1.13 and 0.05 to 0.24 mg/kg, respectively (Mehta *et al*., 9). The lower residue concentrations in onion bulbs compared to potatoes in the present investigation could be attributed to the multiple scales in the onion bulbs due to which only a part of CIPC might have entered inside the bulbs.

In pre- as well as postharvest application of CIPC in *rabi* season, there was no significant difference in the sprouting of the treated samples over the control irrespective of the varieties (data not given). However, in *kharif* season the application of CIPC was found to control sprouting significantly during storage, in cultivar and sprouting was found to be directly correlated to the duration of storage. After three months of storage the sprouting in B. Raj was reduced to 5.36% (2% CIPC at 75 DAP) due to CIPC application as compared to 18.91% in control (Fig. 4). Furthermore, all treatments except 1% CIPC at 90 DAP were found effective to



T1: CIPC spray @ 1%; T2: CIPC spray @ 1.5%; T3: CIPC spray @ 2%

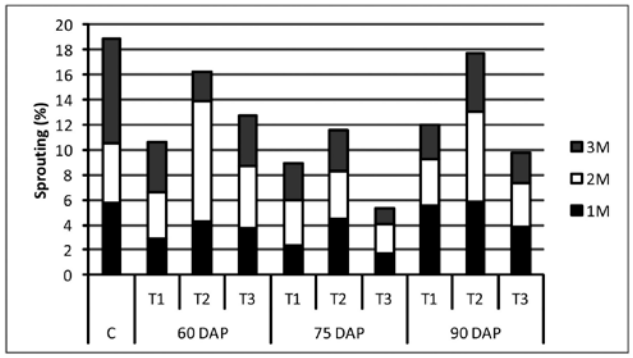
Different lowercase letters on the top of columns represent significant difference at $p \leq 0.05$.

Fig. 3. CIPC residues in different onion varieties after preharvest application in *kharif* season.

control sprouting in B. Red (Fig. 5). Application of CIPC at 2% after 75 DAP was found to be the best treatment: after three months of storage the sprouting in B. Red was reduced to 13.06% in CIPC sprayed crop as compared to 37.43% in control.

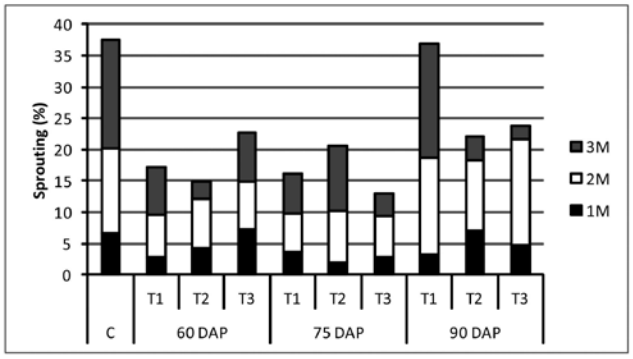
The results showed that the bulb sprouting was effectively suppressed by CIPC in harvest of *kharif* crop only. CIPC is a synthetic compound that modifies spindle formation, inhibits mitosis and prevents sprouting (Vaughn and Lehnen, 16). Further, the arresting of sprouting was also attributed to altered respiration rate due to carbamate isopropyl N-phenyl (CIP) (Benkeblia, 2). It is evident that there is a lack of physiological dormancy phase in the *kharif* crop and the sprouting continues until harvest; unlike *rabi* crop (Shinde *et al*., 13). Further, the low level of residues at 90 DAP treatment compared to 75 DAP treatment revealed that the crop has differential capacity for maximum sequestration of externally applied chemicals *vis-á-vis* physiological maturity. The envisaged suppressant might have reached the deeply situated sprout before initiation of dormancy processes and could have arrested the sprout growth. However, during *rabi* season the physiological dormancy advances simultaneously with the maturity (Shinde *et al*., 13). Therefore, the leaf primordial which has already undergone dormancy fully or partially could not have been affected by CIPC. Similar is the case with the postharvest application, wherein the well cured bulbs were perhaps already in dormancy.

It may be concluded that the application of a growth suppressant such as CIPC in optimum concentration is crucial at a stage: before the crop has entered the physiological dormancy stage. However, further research is needed to quantify the actual dosages *vis-á-vis* spray formulations for even distribution with the time of application. Further the role of CIPC in forced ripening of the *kharif* crop may also be investigated.



C : control; T1 : CIPC @ 1%; T2 : CIPC @ 1. 5%; T3 : CIPC @ 2% Significant difference at $p \leq 0.05$ between control and treatment with the highest value of sprouting

Fig. 4. Effect of preharvest CIPC application on sprouting of onion cv. B. Raj during *kharif* season.



C : control; T1 : CIPC @ 1%; T2 : CIPC @ 1.5%; T3 : CIPC @ 2% Significant difference at $p \leq 0.05$ between control and treatment with lowest value of sprouting

Fig. 5. Effect of preharvest CIPC application on sprouting of onion cv. B. Red during *kharif* season.

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