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Persistence of fipronil and its risk assessment on cabbage, *Brassica oleracea* var. capitata L.

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ABSTRACT

Persistence of fipronil in cabbage was studied following three applications of Jump 80 WG at 75 and 150 g a.i. ha^{-1} at 7 day interval. The average initial deposits of total fipronil (fipronil and its metabolites) were 1.226 and 2.704 mg kg^{-1} on the heads following 3rd application of fipronil at single and double the dosages, respectively. Desulfinyl was found to be the main metabolite followed by sulfone and sulfide. Metabolite amide was not detected in cabbage samples. Half-life periods for fipronil were found to be 3.43 and 3.21 day at single and double the application rates, respectively. Risk assessment of fipronil to the consumers was calculated on the basis of per capita 80 g consumption of cabbage and comparing it to its ADI for an adult of 55 kg which was found to be less than its ADI on 10th day at both the dosages.

1. Introduction

India is known to be the second largest producer of vegetables after China. It produces 13 percent of the world's vegetable output, in which cabbage (*Brassica oleracea* var. capitata) occupies the second position of the total global production of vegetables. In India, the area under cabbage was 265.4 thousand hectares with a production of 5887.8 thousand tones (Anonymous, 2009). In Punjab, the area under cabbage was 3.34 thousand hectares with a production of 73.23 thousand tones (Anonymous, 2008). The low production of cabbage in the country could be attributed to several factors, the most important being the damage caused by various insect pests. Amongst these, diamond back moth (DBM), *Plutella xyloptella* (L.) is the most devastating and cosmopolitan pest of cruciferous vegetables. Its resistance to many of the commonly used insecticides make it one of the most difficult pests to manage (Bharadwaj et al., 2005).

Fipronil[5-amino-1-(2,6-dichloro-α,α,α-trifluoro-p-tolyl)-4-trifluoromethylsulfinyl pyrazole-3-carbonitrile] is a member of phenyl pyrazole class of insecticide first synthesized by Rhône Poulenc Ag Company (now Bayer CropScience) in 1987 and marketed the product in 1993 (Fig. 1) (Tomlin, 2000). It acts on gamma amino butyric acid (GABA) receptors, the principal nerve transmitter of insects, preventing the inhibition of GABA (Sammelson et al., 2004). Fipronil is labeled for use in large number of crops and is effective against a wide range of insect pests. It has been evaluated against over 250 insect pests and on more than 60 crops worldwide (Anonymous, 2004). Fipronil is an extremely active molecule, often requiring only a few grams of active ingredient per hectare to control piercing/sucking and chewing insect pests that are resistant to other agents such as pyrethroids, organophosphates and carbamates (Bobe et al., 1997). In India, Fipronil is marketed under the trade name Regent, Termidor and Jump. The compound controls a broad spectrum of damaging insects and can effectively be delivered to the target pests *via* soil, foliar, bait or seed treatment and is widely used to control many species of soil and foliar insects on various crops such as rice, vegetables and fruits (Tomlin, 1994; Bobe et al., 1998a). It is found to be effective against the control of various insect pests of vegetable crops like chilli, onion and okra especially the thrips (Jadav et al., 2004). Fipronil has been found effective for the management of DBM on cauliflower (Bharadwaj et al., 2005), shoot and fruit borer in brinjal (Sahu et al., 2004) and thrips and mites in chillies (Reddy et al., 2005).

Fipronil degrades to its major metabolities by reduction to sulfide MB 45950 (Ramesh and Balsubramanian, 1999a), oxidation to sulfone MB 46136 (Bobe et al., 1998a), hydrolysis to amide RPA 20076 (Bobe et al., 1998b; Ngim and Crosby, 2001) and photolysis to desulfinyl MB 46513 (Hainzl and Casida, 1996). The desulfinyl photodegrade is extremely stable and actually more

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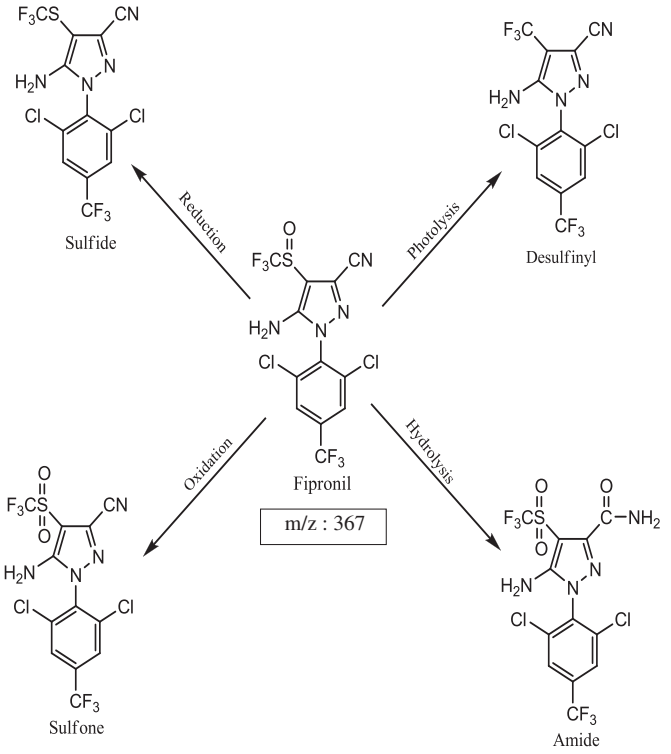

Fig. 1. Metabolites or degradation products of fipronil.

toxic than the parent compound (USEPA, 1996). There are four main degradation products of fipronil and some of the metabolites are more toxic than the parent compound. The metabolite MB 46513 is about 10 times more acutely toxic to mammals than fipronil itself. The metabolites MB 46136 and MB 45950 are more toxic to freshwater invertebrates than fipronil (Pesticide Action Network—UK (PAN), 2000).

In general, pesticide analyses are performed in raw agricultural commodity, which include the peel and (other) non edible parts. However, cabbages are subjected to some form of household preparations, e.g., washing, cooking, removal of non edible parts, etc. before actual consumption. Limited studies have shown that certain types of postharvest treatments or household preparations may help to reduce pesticide residues (Ramesh and Balasubramanian, 1999b; Dhiman et al., 2006).

However, considerable concern is being expressed over the magnitude of pest control chemicals left in food stuffs following their use on crops. It is well recognized, that there are risks associated with the consumption of pesticide-treated crops because of the presence of toxic residues on them. Therefore, the rational recommendation of a pesticide requires that it must not only provide an effective control of pests, but at the same time its residues on the commodity must also be toxicologically acceptable. These residues, if present in excess, may prove hazardous to the health of the consumers. It is important to ensure that the levels of harvest time residues of pesticides on food stuffs do not pose any hazard to consumers and are acceptable in domestic as well as international trade. Therefore, the present studies were undertaken to know the persistence of fipronil and its metabolites on cabbage under sub-tropical conditions of Punjab, India. Information on the nature and amounts of toxicological active metabolites of fipronil in cabbage is necessary to ensure the safety of the consumers and the environment. To reduce the risk of these residues, certain remedial measures need to be taken so that the consumers may safeguard their health while consuming pesticide treated commodities. So another objective of present studies was to study the effect of processing like washing and boiling on the reduction of fipronil residues on cabbage.

2. Materials and methods

2.1. Chemicals and reagents

The technical grade analytical standards of fipronil MB-46030 (purity 97.5 percent), sulfone MB-46136 (purity 99.7 percent), sulfide MB-45950 (purity 98.8 percent), desulfinyl MB-46513 (purity 97.8 percent) and amide RPA-20076 (99.8 percent) were supplied by M/s Bayer CropScience India Ltd., Mumbai, India. Fipronil (Jump 80 WG) formulation was used for field application and was also obtained from M/s Bayer CropScience, Mumbai, India. Analysis of acetone extract of the formulation showed the presence of fipronil, and none of its metabolic products and no interfering peak was observed in the vicinity of the retention time of the compounds being studied. Moreover, the concentration of fipronil was found to be accurate with respect to its purity as claimed by the manufacturers.

Solvents like acetone, dichloromethane and hexane were procured from Merck, Darmstadt, Germany. Sodium chloride (ASC reagent grade $\geq$ 99.9 percent) was also obtained from Merck, Darmstadt, Germany. Sodium sulfate anhydrous (AR grade) was from S.D. fine Chemicals, Mumbai. All common solvents were redistilled in all-glass apparatus before use. The suitability of the solvents and other chemicals was ensured by running reagent blanks before actual analysis.

2.2. Preparation of standard solution

A standard stock solution of the parent compound fipronil and its metabolites (1 mg/mL) was prepared in acetone. The standard solutions required for constructing a calibration curve (2.00, 1.50, 1.00, 0.50, 0.25 and 0.10 $\mu g\,mL^{-1}$) were prepared from stock solution by serial dilution with acetone. All standard solutions were stored at 4 °C before use.

2.3. Instruments

Analysis of the fipronil and its metabolites was carried out on gas liquid chromatography (GLC, Clarus 500) equipped with electron capture detector (ECD) ^{63}Ni supplied by M/S Perkin Elmer, Switzerland. A capillary column Rtx-5 (30 m $\times$ 0.53 mm i.d. $\times$ 0.25 µm film thickness of 5 percent phenyl 95 percent methyl polysiloxin) with split ratio 1:10 was used for estimation of fipronil and its metabolites. Confirmation of fipronil and its metabolites were carried out on a GC (Shimadzu 2010) coupled with mass detector (Fisons MD-800, quadrupole mass detector) equipped with capillary column (GCMS-QP 2010 plus, Shimadzu, Rtx-5 Sil MS). A capillary column (30 m $\times$ 0.25 mm i.d. $\times$ 0.25 µm film thickness) was used for confirmation of these residues of fipronil and metabolites. The system software used was GCMS solution version 2.5.

2.4. Field trials

2.4.1. Crop planting for the field experiment

Cabbage (var. Drum Head Late) was raised during November 2010 to April 2011 at Entomological Research Farm, Punjab Agricultural University, Ludhiana following recommended agronomic practices (Anonymous, 2008). There were three replications for each treatment (i.e. control, recommended and double the recommended dosages) the recommended dosages arranged in a randomized block design (RBD), and size of the each plot was 100 m^2.

2.4.2. Application of the insecticide

The first application of fipronil (Jump 80 WG) at 75 and 150 g a.i. ha^{-1} was made at head formation stage followed by another two applications at 7 day interval in all the treatment plots with the help of a knapsack sprayer fitted with hollow cone nozzle. In control plots, only water was sprayed.

2.4.3. Sampling procedure

About 5 to 6 marketable size cabbage head samples were collected randomly from control and treated plots of each treatment at 0 (2 h), 1, 3, 5, 7, 10 and 15 day after application of the insecticide. The samples from each treatment plot were pooled and mixed thoroughly on a sheet of polyethylene in the field and a subsample of 1 kg was drawn after chopping and quartering, packed in polyethylene bags and brought to laboratory for immediate extraction and cleanup.

In the laboratory the sub-samples belonging to 0 day and 1 day were divided into three parts, one part was macerated in the blender and a representative 50 g sample was subjected to extraction and cleanup as given under Section 2.5. Second part was subjected to household process of washing under tap water for