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Bioefficacy, phytotoxicity, safety to natural enemies and residues of flubendiamide in sugarcane (*Saccharum* spp. L.) under field conditions

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ABSTRACT

Sugarcane early shoot borer, *Chilo infuscatellus* Snellen is a major menace and cause of considerable damage to sugarcane crops in India. The incidence of early shoot borer has been increasing for the last few years. A new insecticide flubendiamide was evaluated in the field to assess its bioefficacy against sugarcane early shoot borer during February to December 2013 and August 2013 to July 2014. Two rounds of foliar application of flubendiamide 20 WG at 60 and 50 g a.i. ha^{-1} significantly reduced the early shoot borer in both experiments. Foliar application of flubendiamide 20 WG (50, 100 and 200 g a.i. ha^{-1}) in sugarcane did not cause any phytotoxic symptoms. Flubendiamide 20 WG test doses were found to be safe to natural enemies including spiders and coccinellids in the field. Though there was a short term decline in the natural enemy population, it started increasing gradually within a fortnight after treatment. The sugarcane yield increased over the control in the flubendiamide 20 WG (60 g a.i. ha^{-1}) treated plots by 32.7 and 31.5% in the two seasons, respectively. The residues of flubendiamide at 50 and 100 g a.i. ha^{-1} were below detectable levels in sugarcane leaf, juice and soil at harvest.

1. Introduction

Sugarcane is an important commercial crop in India and provides a livelihood to more than five million farm families. India is the second largest producer of sugarcane contributing about 19.6 per cent of the canes produced in the world. Although India occupies 2nd place in terms of area and production of sugarcane in the world, the productivity has been stable around 67.5 ± 3 t ha^{-1} over the past two decades. Among several constraints in increasing the productivity, the loss due insect pests is of great importance and it needs to be addressed adequately by employing novel approaches. In India, more than 200 insects were recorded as pests of sugarcane though only 18 of them are considered as major pests. The Crambid borers of sugarcane *viz*., early shoot borer *Chilo infuscatellus* Snellen, internode borer *Chilo sacchariphagus indicus* (Kapur), root borer *Emmalocera depressella* Swinhoe and the stalk borer *Chilo auricilius* Dudgeon are the major pests responsible for considerable yield loss every year. Hence, management of these borers through economically viable and environmentally benign approaches has become necessary inevitable to break the plateau in productivity.

Various strategies have been practiced for controlling the borers in sugarcane. Insecticides are affording significant results initially and hence chemical control methods are the first line of defense for managing these pests. Most of the insecticides used on agricultural crops belonging to organophosphates, carbamates and synthetic pyrethroid groups which are referred to as conventional insecticides hereafter, showed that a certain amount of residues were present at detectable limits.

In recent times, new insecticide molecules have provided advantages over earlier chemistry chemicals in terms of high toxicity to insects at lower doses, greater levels of safety, better performance and reduced environmental impact. One such new insecticide is flubendiamide, which has shown outstanding efficacy against borers. Flubendiamide (NNI-0001) is a new insecticide and globally co-developed by Nihon Nohyaku Co., Ltd. and Bayer Crop Science AG. The parent structure of flubendiamide was originally discovered by Nihon Nohyaku during a herbicide program on pyrazine dicarboxamides in 1993, and the discovery of more potent substituent's led to the synthesis of the insecticide flubendiamide in 1998 (Tsubata et al., 2007).

The introduction of the new compound flubendiamide on to the market was in July 2005 (Nishimatsu et al., 2005) and the first registration was obtained in the Philippines in 2006, followed by

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Japan, Pakistan, Chile, India and Thailand in 2007. Flubendiamide is a novel class insecticide of the chemical family of 1,2-benzenedicarboxamides or phthalic acid diamides. Flubendiamide acts by selective activation of the ryanodine receptor (RyR) in insects, inducing ryanodine-sensitive cytosolic Ca^{2+} transients independent of the extracellular Ca^{2+} concentration (Ebbinghaus et al., 2006; Nauen, 2006). The ryanodine-sensitive intracellular Ca^{2+} release channels (commonly called ryanodine receptors) are intracellular. The function of these specialized channels is the rapid and massive release of Ca^{2+} from intracellular stores, which is necessary for contraction of muscles. In the case of flubendiamide, the compound disrupts calcium regulation by an allosteric mechanism.

Newer molecules often have a higher stability and superiority over conventional pesticides to control the pest population in a classical manner at field level. In this array, the new group (phthalic acid diamide molecules) flubendiamide was discovered as a novel class of insecticide having a unique chemical structure; confirmed excellent activity against broad spectrum of lepidopterous pests and also has an excellent environmental profile with low impact on fish, birds and mammals. Further, the compounds exhibited little or no toxicity to many common natural enemies, beneficial arthropod species, and pollinators (Anonymous, 2009). The high degree of mammalian safety, relatively low use rates compared to standard insecticides (eg. organophosphates), long residual properties, and broad spectrum activity against lepidopteran pests made diamides an excellent control option in pest management systems. Biological activity of flubendiamide against various lepidopterous pests evaluated under laboratory conditions in Japan revealed that flubendiamide was highly active against important lepidopterous pests (Tohnishi et al., 2005). Flubendiamide is sold under the trade names Amoli®, Belt®, Fame®, Fenos®, Phoenix® and Takumi® (Hirooka et al., 2007).

Flubendiamide 20 WG at 25, 35 g a.i. ha^{-1} controlled rice stem borer, *Chilo suppresalis* Walker and leaf folder, *Cnaphalocrocis medinalis* Guen. (Mallikarjunappa et al., 2008). Chatterjee and Mondal (2012) observed that, flubendiamide 20 WG effectively checked the mean fruit damage (3.5%) which was reflected on the highest yield (81.5 q ha^{-1}) in tomato. Flubendiamide 480 SC at 100 ml ha^{-1} caused significant reduction in *Helicoverpa armigera* Hub. and *Maruca testulalis* (L.) larvae, and recorded minimum flower and pod damage (Ameta et al., 2011). Tatagar et al. (2009) tested flubendiamide 20 WG at 60 g a.i. ha^{-1} against fruit borers, *H. armigera*, *Spodoptera litura* (Fab.) and reported the lowest fruit damage with chilli yield of 7.48 q ha^{-1}. Similarly, flubendiamide 480 SC at 48 g a.i. ha^{-1} recorded considerable reduction in larval population of *Plutella xylostella* (L.) in cabbage (Vinothkumar et al., 2007). However, there are no reports on *in-vivo* and field evaluation of flubendiamide 20 WG against the *C. infuscatellus* on sugarcane. In this context, the present investigation was undertaken with the objectives to evaluate the bioefficacy, phytotoxicity, safety to natural enemies and residues of flubendiamide 20 WG in sugarcane ecosystem.

2. Materials and methods

2.1. Chemicals and reagents

The Commercial formulation of Flubendiamide 20 WG and analytical standards of flubendiamide with 98.28 per cent purity were obtained from Rallis India Ltd., Bengaluru, India. Acetonitrile, ethyl acetate, hexane of analytical HPLC grade; sodium chloride, anhydrous magnesium sulphate and Primary Secondary Amines were purchase from Sisco Research Laboratories Pvt. Ltd., Mumbai, India.

2.2. Preparation of standard solution

A standard stock solution of 1000 μg ml^{-1} was prepared by dissolving 101.75 mg of flubendiamide technical standard (98.28% purity) in 100 ml of acetonitrile (HPLC grade). An intermediate standard solution of 100 μg ml^{-1} was prepared by diluting 10 ml of stock solution in 90 ml of acetonitrile in a 100 ml volumetric flask. Working standard solutions of 0.05, 0.10, 0.50 and 1.0 μg ml^{-1} were prepared by diluting 10 ml of the intermediate standard solution sequentially with 90 ml of HPLC grade acetonitrile which were used for spiking and calibration.

2.3. Field experiments

Supervised field experiments were conducted at Tamil Nadu Agricultural University, Coimbatore, India for two seasons. First season field trial conducted with sugarcane cv. CO 95025 under irrigated condition during February 2013 to December 2013 and second season during August 2013 to July 2014 with cv. CO 86032 in a randomized block design with eight treatments and replicated thrice. Sugarcane was raised with 60 m^2 plots following standard agronomic practices except insect pest management. The treatments for the management of early shoot borer comprised Flubendiamide 20 WG @ 40, 50 and 60 g a.i. ha^{-1} along with Chlorantraniliprole 18.5 SC @ 75 g a.i. ha^{-1}, Chlorpyrifos 20 EC @ 250 g a.i. ha^{-1}, Thiodicarb 75 WP @ 675 g a.i. ha^{-1} and Fipronil 5 SC @ 75 g a.i. ha^{-1}. An untreated control was simultaneously maintained during the study. Treatments were applied using a knapsack hand sprayer equipped with a hallow cone nozzle. Foliar spraying was done at 30 day intervals starting from the 30^{th} day after planting at a spray volume of 500 l ha^{-1}.

2.3.1. Bioefficacy of flubendiamide 20 WG against Chilo infuscatellus in sugarcane

The number of plants with early shoot borer damage and total number of plants in randomly selected pairs of rows were recorded per plot leaving border rows before and 10, 20, 30 days after each spray and per cent damage was calculated. Sugarcane yield $plot^{-1}$ was recorded at the time of harvest and the total yield was expressed in t ha^{-1}. The yield data was subjected to statistical analysis and the percentage increase over the control was determined.

2.3.2. Phytotoxicity of flubendiamide 20 WG on sugarcane

Phytotoxicity caused by flubendiamide 20 WG on sugarcane was evaluated in field experiments and the treatments used in bioefficacy studies were used along with double the recommended dose of 50 g a.i. ha^{-1} and was replicated three times. Five plants were selected at random in each plot and the plants were examined for symptoms of phytotoxicity *viz.*, leaf injury, wilting, vein clearing, necrosis, epinasty and hyponasty on 1, 3, 5, 7, 10, and 14 days after treatment. The per cent leaf injury was calculated using the following formula,

$$\text{Per cent leaf injury} = \frac{\text{Total grade points}}{\text{Maximum grade} \times \text{No. of leaves observed}} \times 100$$

The phytotoxicity symptoms, were graded based on per cent injured as prescribed by Central Insecticide Board and Registration Committee (CIBRC, India) grade scale. The 0–100% scale is subdivided in to 10 equivalent grades 0 to 10 *viz.*, no phytotoxicity grade 0; 1–10% grade 1; 11–20%...90–100% grade 10.