

## Effects of sublethal concentration of Imidacloprid on the enzyme activity of sweet potato weevil, *Cylas formicarius* (Fabricius) (Coleoptera, Brentidae)

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**ABSTRACT:** Application of sublethal (LC<sub>10</sub> and LC<sub>30</sub>) dose of Imidacloprid on sweet potato weevil was found to have inhibitory effect on its enzymes viz., glutathione reductase (GR), glutathione peroxidase (GPx), reduced glutathione (GSH) and glutathione S-transferase (GST), while activity of superoxide dismutase (SOD) and lipid peroxidase (LPx) was up regulated when compared to control. The weevil's expression of SOD increased by 13.53 and 69.44 and LPx by 67.38 and 73.04 per cent respectively, when the sublethal dose was raised from LC<sub>10</sub> to LC<sub>30</sub>. Although GST and GPX did not alter considerably after exposure to the sublethal doses of imidacloprid, weevil activity of GR (65.5-78.1%) and GSH (42.2 and 61.6%) decreased significantly. © 2024 Association for Advancement of Entomology

**KEY WORDS:** Glutathione reductase, glutathione peroxidase, reduced glutathione, glutathione S-transferase, superoxide dismutase, lipid peroxidase

### INTRODUCTION

Sweet potato, (*Ipomoea batatas*), as it is grown by subsistence farmers, is known as the “poor man’s crop”, and it is ranked as the seventh largest food crop in the world after wheat, rice, maize, potato, barely and cassava (Narayan *et al.*, 2022). Cultivation of sweet potato is prevalent in all most the states of India; however, majority of the nation’s supply is from Odisha, Kerala, West Bengal, and Uttar Pradesh (Palaniswami *et al.*, 1991; Prakash *et al.*, 2020). Sweet potato weevil (SPW), *Cylas formicarius* (Fabricius) (Coleoptera, Brentidae), is considered to be the deadliest insect pest, inflicting significant damage to sweet potato tubers that can

occasionally reach 100 per cent (Palaniswami and Chattopadhyay, 2005; Prasad *et al.*, 2022). Grub of SPW excavates tunnels and feeds, while the adult feeds petioles and leaves. Chemical pesticides have historically been used to suppress SPW (Palaniswami and Mohandas, 1996; Zhang *et al.*, 2013). When the neonicotinoid imidacloprid comes in contact with an insect pest, its central nervous system is damaged and its nicotinic acetylcholine receptors are disturbed (Jeschke *et al.*, 2011; Le Goff and Giraud, 2019). According to Elbert *et al.* (2008) Imidacloprid has a systemic action that helps to a variety of piercing-sucking insect pests, chewing pests, and soil-dwelling arthropods.

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Neonicotinoid pesticides impact the lifespan, feeding activity, larval duration, reproduction, and activity of the detoxifying enzymes of exposed insects at sublethal doses (Tan *et al.*, 2012; De Franca *et al.*, 2017). Insects may occasionally develop resistance to insecticides when subjected to sublethal concentrations of these chemicals. Sublethal doses of insecticides have an impact on the activity of detoxifying enzyme in a variety of insects (Jing *et al.*, 2011; He *et al.*, 2013; Lu *et al.*, 2016); nevertheless, the literature review did not yield the same as in the case of *C. formicarius*. The current work ascertains that the sublethal exposure of imidacloprid to SPW activates several enzymes, including super oxide dismutase, lipid peroxidase, but reduces the activity of glutathione peroxidase, glutathione reductase, glutathione S-transferase and reduced glutathione.

## MATERIALS AND METHODS

Sweet potato tubers infected with SPW collected from the markets and fields of the ICAR-Central Tuber Crops Research Institute (CTCRI), Thiruvananthapuram were stored in one-litre plastic containers. Muslin cloth was used to cover the container's mouth. The container was filled with newspaper scraps to absorb the water that was released by tubers. As the adults emerged, they were collected, and their culture was maintained on fresh tubers (at 32°C and 75% RH).

Imidacloprid was diluted to five different concentrations 0.0001, 0.0005, 0.001, 0.005, 0.01, 0.05, 0.1 and 0.5 per cent in ordinary water. Using a micro applicator, 50µl of the aliquot was topically delivered to each of the 20 adults of 2-week-old. Three replications were kept for each treatment. In the control group, Imidacloprid was replaced with water. Mortality of the weevil was observed 24 hours after treatment (HAT), and LC<sub>10</sub>, LC<sub>30</sub> and LC<sub>50</sub> were calculated using Probit regression analysis (Finney, 1971).

**Assessment of enzyme activities:** The activity of six detoxifying enzymes *viz.*, superoxide dismutase (SOD), lipid peroxidase (LP), glutathione reductase (GR), glutathione peroxidase (Gpx),

reduced glutathione (GSH) and glutathione S-transferase (GST) were identified for the current investigation.

**SOD** The activity of SOD was assayed by the procedure adopted by Misra and Fridovich (1977).

**LPx** assay is based on the reaction of Malondialdehyde (MDA) with of Thiobarbituric acid (TBA) forming an MDA-TBA adducts (Ohkawa *et al.*, 1979).

**GR** was assayed by the procedure adopted by David and Richard (1983).

**GPx** catalyses the oxidation of reduced glutathione (GSH) to oxidized form which reacts with Nicotinamide Adenine Dinucleotide Phosphate (NADPH) and gets converted to NADP and two molecules of reduced glutathione which is measured by spectrophotometer at 340 nm (Wendel, 1980).

**GSH** was estimated as described by Moron *et al.* (1979).

The activity of GST in treated test insects were assayed by the procedure adopted from Mannervik (1985).

The total protein content of the SPW estimated by Lowry's method (Lowry *et al.*, 1951) using bovine serum albumin (Sigma) as a standard.

Data were subjected to analysis of variance (ANOVA) using SPSS version 17. The mean values of data were tested with Fisher's Least Significant Difference (LSD) multiple comparison tests were performed to assess the significance of Imidacloprid effects on enzyme activity (P<0.05).

## RESULTS AND DISCUSSION

**Bioassay and determination of sublethal concentrations:** Mortality of adult SPW increased with an increase in the concentration of imidacloprid. Exposure at lethal and sublethal doses revealed the LC<sub>50</sub> to be 0.001 ml L<sup>-1</sup>, whereas the LC<sub>10</sub> and LC<sub>30</sub> were 0.0001 and 0.0006 ml L<sup>-1</sup>, respectively; and these concentrations were used for further studies.

**Effect of sublethal concentrations on enzyme activity:** Adult SPW showed a substantial ( $P < 0.001$ ) variation in SOD activity between the treatment and control batches following the sublethal doses of imidacloprid administration. The SOD activity of SPW dramatically increased from its control value of  $16.62 \pm 0.03$  to  $18.39 \pm 0.20$  and  $28.16 \pm 0.47$ , respectively, after being treated with imidacloprid at  $LC_{10}$  and  $LC_{30}$  (Fig. 1a). Sublethal concentrations of imidacloprid significantly ( $P < 0.001$ ) up regulated the activity of lipid peroxidation in SPW (Fig. 1b). The MDA unit in the untreated batches of SPW was recorded to be  $75.40 \pm 0.90 \text{ mg}^{-1} \text{ protein}$ , but it significantly increased to  $231.21 \pm 0.70$  and  $279.7 \pm 0.8 \text{ mg}^{-1} \text{ protein}$  in the treatments of  $LC_{10}$  and  $LC_{30}$  of imidacloprid. Activity of the GPx in SPW was found significantly ( $P < 0.001$ ) varied in the treatment with imidacloprid (Fig. 1c). The enzyme  $\text{mg}^{-1} \text{ protein}$  in the untreated SPW was  $1.56 \pm 0.04$ , whereas it decreased to  $0.29 \pm 0.01$  and  $0.14 \pm 0.01$ , respectively in the treatments with  $LC_{10}$  and  $LC_{30}$  concentrations of imidacloprid. The activity of GR was estimated by assessing the amount of NADPH utilized by the enzyme to produce reduced glutathione. The oxidation of NADPH in the untreated SPW was  $1.89 \pm 0.10 \mu\text{moles mg}^{-1} \text{ protein}$ , whereas it was significantly decreased to  $0.37 \pm 0.03$  in  $LC_{10}$  and  $0.45 \pm 0.02 \mu\text{moles}$  in  $LC_{30}$  concentrations of imidacloprid; however, this variation was statistically not significant ( $P < 0.001$ ) (Fig. 1d). A decrease in the GST's detoxifying activity was observed in SPW when treated with sublethal concentration of Imidacloprid than the control (Fig. 1e). In the case of untreated batch of SPW, the enzyme activity was  $3.25 \pm 0.01 \text{ mg}^{-1} \text{ protein}$ , whereas it was  $0.18 \pm 0.01$  and  $0.17 \pm 0.01$ , respectively when treated with  $LC_{10}$  and  $LC_{30}$ . The GSH level was significantly ( $P < 0.001$ ) decreased in the treated insects (Fig. 1f). In the case of untreated SPW the level of GSH was  $654.67 \pm 0.36$  units  $\text{mg}^{-1} \text{ protein}$  and it was  $480.56 \pm 0.38$  and  $145.07 \pm 0.54$ , respectively when treated with  $LC_{10}$  and  $LC_{30}$  concentration.

Insects are exposed to a variety of xenobiotic toxins throughout their lives; some are made by plants in their natural condition, such as allelochemicals, while

others take in the form of insecticides. In spite of this, insects have developed a wide range of detoxification strategies to fight the natural poisons. In some circumstances, the same mechanisms help insects resist insecticides; although the extent and type of processes vary significantly. Understanding detoxification enables one to decipher agricultural plants' chemical defence mechanisms and to choose more effective insecticides. Detoxifying enzymes play a vital role in the insect resistance mechanisms, and a variation in their activities can be seen during insecticide metabolism (Feng *et al.*, 2018; Jin *et al.*, 2019). Reactive oxygen species (ROS), which are produced when synthetic insecticides are applied, can cause oxidative stress in insect cells. SODs are ubiquitous enzymes that serve as an organism's first line of defence against oxygen free radicals. According to Yamamoto and Yamaguchi (2022) SOD can shield healthy cells from ROS and eliminate superoxide radicals ( $O_2^-$ ) through the process of dismutation to oxygen and hydrogen peroxide. Imidacloprid treatment at the two sublethal concentrations enhanced the activity of SOD of SPW (13.53 and 69.44%, respectively), when compared to the control. The increased rate of SOD indicates the detoxification of imidacloprid in SPW by removing the superoxide radicals ( $O_2^-$ ) through the process of dismutation to oxygen and hydrogen peroxide. An increased level of SOD is an indicative of SPW's attempt to respond to an oxidative stress condition. Elevations of SOD due to the exposure of imidacloprid in different species have already been reported in insects as well as mammals. These were reports by El Gendy *et al.* (2010) in male mice, Kapoor *et al.* (2010) in rat, Sun *et al.* (2015) in *Coloana cinerea*, Yang *et al.* (2015) in *Harmonia axyridis*, Zhu *et al.* (2015) in *Aphidius gifuensis*, Wang *et al.* (2016) in *Ambrostoma quadrimopressum* and Balieira *et al.* (2018) in *Apis mellifera*. Whereas some studies show the inhibitory effect of imidacloprid stress in some insects. Zhou *et al.* (2017) noted a down regulation in SOD level in *Aphidius gifuensis* and Zhang *et al.* (2020) in *Frankliniella occidentalis* and *F. intonsa* when treated with Imidacloprid. Kolawole *et al.* (2014) explained this was due to the limited efficiency of SOD in some species to scavenge the accumulated  $O_2$  radicals

in cell on prolonged treatment of insecticides.

Increased lipid peroxidation is a sign of the oxidative breakdown of cell membrane lipids, which results in cell damage under pesticide stress. According to Gawel *et al.* (2004) the presence of malondialdehyde (MDA) is a sign of LPO and, subsequently, oxidative stress. Imidacloprid exposure caused the lipid peroxidation rate in SPW to increase it by 3.3 and 5.4 times, respectively, compared to untreated batches. El-Gendy *et al.* (2010), Kapoor *et al.* (2010), Bal *et al.* (2012), Balieira *et al.* (2018), and Ndonwi *et al.* (2019) reported that imidacloprid treatment increases the concentration of MDA in various animal tissues. Gauthier *et al.* (2018) found that imidacloprid and thiamethoxam treatment increased lipid peroxidation in susceptible *Apis mellifera*. The presence of a significant amount of LPO in the treated SPW tissue indicated that the metabolism of imidacloprid resulted in the production of

oxidative metabolites or free radicals, which may have the potential to cause progressive chain reactions. Lipid peroxidation has a pivotal role to determine the longevity of insects, when it rises above the critical level, it may culminate into the death of the insect; however, if it falls below the threshold level, the insect may live longer (Gawel *et al.*, 2004).

GSH and GPx support cellular defence by eliminating membrane phospholipid hydroperoxides. Members of the glutathione peroxidase (GPx) family play a critical role in antioxidant defense by converting organic hydroperoxides and/or hydrogen peroxide to water and/or their corresponding alcohols (Masella *et al.*, 2005). The current investigation revealed that GPx, a crucial mechanism of pesticide resistance, dropped to between 81 and 91 per cent when SPW was subjected to sublethal doses of imidacloprid, which

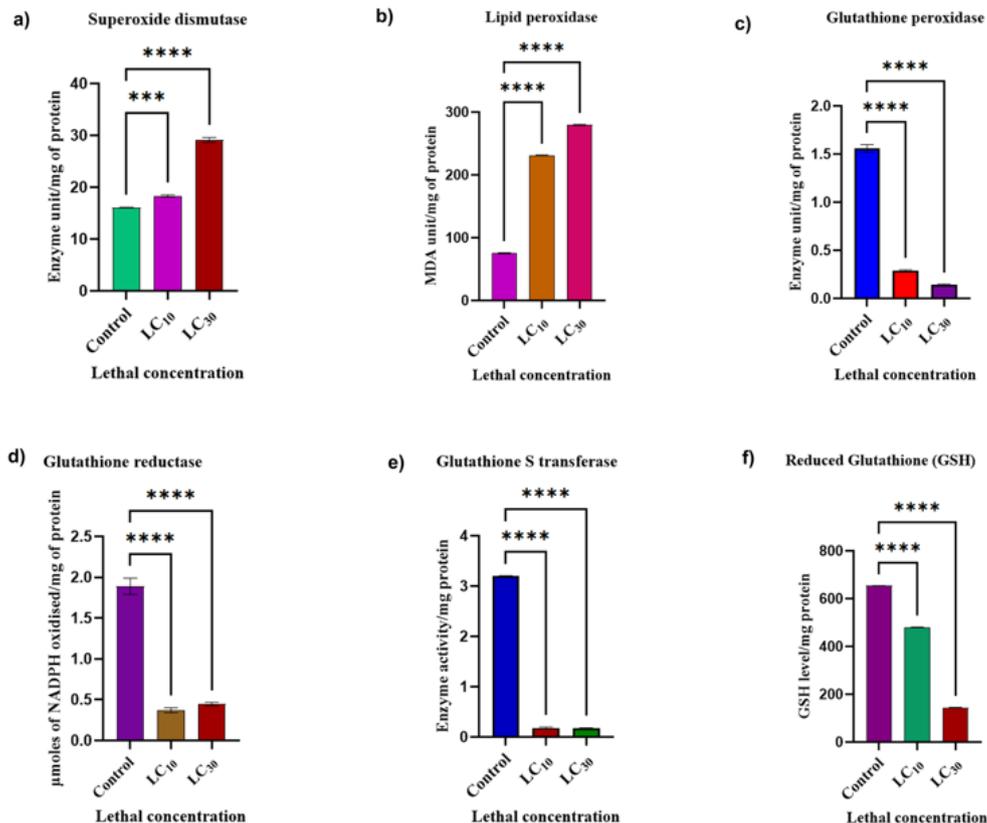


Fig. 1 Activities of detoxifying enzymes a- SOD, b- LPx, c- GPx, d- GR, e- GST, f- GSH in *Cylas formicarius* on the exposure to sublethal concentrations of imidacloprid

shows that GPx has a negative tolerance or resistant to imidacloprid. Contrary to the current findings, exposure to imidacloprid results in oxidative stress and resistance in a variety of different species, such as honeybees (*Apis mellifera*), rats, and mice (El-Gendy *et al.*, 2010). Che-Mendoza *et al.* (2009) reported an increase in the tolerance of mosquitoes against pyrethroids through an elevation in the expression of GPx. Bamidele *et al.* (2017) evaluated the metabolic defence mechanism by administering dichlorvos to African palm weevil larvae (*Rynchophorus phoenicis* Fabricius) found a significant increase in GPx activity.

The glutathione system, GR removes hydrogen peroxide and organic hydroperoxides such as lipid hydroperoxides on pesticide exposure (Maheshwari *et al.*, 2011). After receiving the sublethal dosages of imidacloprid, the GR of SPW was found decreased. The decrease in enzyme activity shows that SPW is vulnerable to imidacloprid. At sublethal dosages of imidacloprid, GR activity in SPW decreased (by 65.5 and 78.1%), indicating that it is less active than the control. Bamidele *et al.* (2017) observed that the activity of GR decreased in response to an increase in the concentration of dichlorvos used to treat *R. phoenicis* larvae. According to Karadag (2019) imidacloprid and thiamethoxam doses ranging from 25 to 500mg L<sup>-1</sup> had no discernible impact on the GR enzyme activities in baker's yeast, *Saccharomyces cerevisiae*.

Glutathione S-transferases (GSTs) are multifunctional enzymes that are responsible for the metabolism and detoxification of both xenobiotic and physiological substances. GSTs can metabolize insecticides by facilitating their reductive dehydrochlorination or by conjugation reactions with reduced glutathione to produce water-soluble metabolites that are simpler to excrete (Hernandez *et al.*, 2018). Imidacloprid treatment at sublethal quantities resulted in GST activity on SPW being 94% lower than control *ie*, the enzyme activity was dropped from 3.25±0.01 mg<sup>-1</sup>protein, to 0.18±0.01 and 0.17±0.01, respectively when treated with LC<sub>10</sub> and LC<sub>30</sub> concentrations. As GSTs play a crucial role in the insecticide resistance, high levels of GSTs

are typically observed in insects that are resistant to pesticides (Perini *et al.*, 2021). Shojaei *et al.* (2017) reported the reduction of GST activity in *Tribolium castaneum* (Herbst) after the treatment with essential oil isolated from *Artemisia dracuncululus*. Several reports show an increased level of GST in insects on the exposure to sublethal concentrations of imidacloprid, this include *Sitobion avenae* (Fabricius) and *Rhopalosiphum padi* (Linnaeus) (Lu *et al.*, 2016) and *Nilaparvata lugens* (Yang *et al.*, 2020). A high level of GST in the resistant strains of *Culex pipiens* treated with organochlorine, organophosphate, and pyrethroids was reported by Mustafa and Ek (2015). According to the current study, GSH levels were found decreased by 42.2 and 61.6 per cent in SPW when it was given sublethal dosages of imidacloprid at LC<sub>10</sub> and LC<sub>30</sub>, respectively. Kapoor *et al.* (2010) ascertained that imidacloprid has produced a significant reduction in the GSH level in female rats. Pyrethroid exposure to a *Nilaparvata lugens* colony in a lab decreased the glutathione and caused an oxidative stress (Vontas *et al.*, 2001). The current study reports that treatment with sublethal concentrations of imidacloprid caused significant impairment in the antioxidant enzyme system of SPW. The activity of SOD and LPx, increased in the treated batches of sweet potato weevil, whereas it reduced as in the case of GPx, GST, GR and GSH. Increased sublethal concentrations of imidacloprid exhibits more oxidative stress in SPW due to the over expression of SOD and LPx, while glutathione related enzymes were down regulated.

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