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REVIEW

Paclobutrazol and Its Use in Fruit Production: A Review

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ABSTRACT

There are documentary records referring to paclobutrazol (PBZ) as a growth bioregulator that inhibits the gibberellin synthesis and its application increases yields in fruit and vegetable crop productions. Its agronomic management includes it as an emerging technology to reduce vigour, promote flower induction and flower development in fruit trees with increased economic returns. Its use is banned in some countries because of concerns about residues that can cause harmful effects on the environment. Therefore, the aim of this article was to collect, analyse and summarise relevant information on the use of PBZ in fruit tree production and its possible risks to the environment. The results obtained indicated that the application of PBZ can be effective in solving some problems related to flowering if it is applied in the right amount and at the right time. However, it is necessary to elucidate the physiological processes with which it is associated and its response to be taken into account to increase yield. PBZ is currently used in fruit trees such as mango, lime, apple and guava, increasing their productivity. However, some studies have shown its residual effect on the environment. Therefore, PBZ is a viable strategy, because it presents a series of advantages in the production of fruit trees. However, it is vital to generate protocols that seek its regulation with a rational and sustainable approach.

KEYWORDS

Environment; flower induction; gibberellin biosynthesis; growth bioregulator; vegetative growth

1 Introduction

Globally, agriculture is one of the most important economic activities, given its contribution to food production [1]. Population growth has a direct impact on the need to increase crop yields, and consumers expect high quality, nutritious, clean, healthy and safe products [2]. The integration of emerging technologies in the different production systems must be in an environment of safety and sustainability [1]. Therefore, the Food and Agriculture Organization of the United Nations (FAO) coined the concept of food security as "physical and economic access to sufficient, safe and nutritious food to meet one's dietary needs and food preferences for an active and healthy life" [3].

The application of nutrients and growth bioregulators increases crop productivity [2]. The latter can be classified according to the physiological processes with which they are associated and their response to application [4]. Previous reports indicate that low concentrations of these compounds facilitate agronomic management, and their effect can be linked to inhibition, slowing and induction of sprouting, flowering and fruit ripening [5]. Among these growth bioregulators is paclobutrazol (PBZ) (growth inhibitor) [6–8], which reduces vigour, and promotes flower induction and development [9,10].

In the last decade, among the common agronomic management practices for fruit tree production is the use of PBZ, which promotes various effects. For example, in India it is used to increase fruit quality in litchi (*Litchi chinensis* Sonn) "China" by inhibiting vegetative growth [11], pear "Clapp's" (*Pyrus pyrifolia* L.) [12] and Indian walnut "Ullal-3" (*Anacardium occidentale* L.) [9]. In China, it is used to reduce vegetative growth in the cultivation of "Western Schley" and "Mahan" pecan [*Carya illinoinensis* (Wang.) K. Koch] [13,14]. In Brazil, it is used to increase the size and shape of the fruit of the "Hass" avocado (*Persea americana* L.) [15].

In Mexico it is used to promote flowering and increase production in guava (*Psidium guajaba* L.) "Calvillo", "Hidrosac" and "Caxcana" [16], and in mango (*Mangifera indica* L.) "Tommy Atkins" [17] "Ataulfo" and "Manila" [18,19].

The use of PBZ contributes to increase production value (yield and fruit quality), however, there is evidence of its residual effects and its negative effect on consumers' health [20]. In addition, there is information that it can cause environmental pollution (groundwater and soil) [21]. The aim of this paper was to collect, analyse and summarise information on the use of PBZ in the production of some economically important fruit trees and their possible risks to the environment.

2 Growth Bioregulators

Among the production costs associated with the application of products to improve and increase the yield and quality of harvested fruit, the application of growth bioregulators constitute a minimal portion [5]. However, in recent years, their application has increased due to the fact that they help to improve the productivity of fruit trees.

Plants synthesise various compounds, including growth bioregulators, which promote and regulate their physiological processes (vegetative growth, flower induction, fruit set and fruit size increase) [7,22,23]. In addition, they influence the response to biotic and abiotic stresses [24]. Traditional bioregulators include auxins, gibberellins, cytokinins, and abscisic acid [25]. Research also considers brassinosteroids, salicylic acid, triazoles, jasmonates, polyamines and recently strigolactones as bioregulators [24,26,27].

Growth bioregulators can be classified according to their mode of action (Tab. 1). It is reported that they can enhance plant defence mechanisms, promote cell division and growth, processes that help to increase yield and quality fruit [28]. They can also delay or inhibit growth [8]. They also play an important role in responses to stress and adaptation such as to water deficit, high temperatures, salinity and flooding [23].

Table 1: Classification and mode of action of growth bioregulators

Classification	Examples	Mode of action	References
Auxin	Indoleacetic Acid, Indole Butyric Acid, and Naphthaleneacetic Acid	Controls cell division, cell expansion (by forming cellulose), apical dominance, tropisms, stem elongation, root formation and development.	[26]
Gibberellins	Gibberelic Acid	Control fruit development (promotes cell division and cell expansion), seed germination, stem elongation, flowering and leaf senescence.	[29,30].

(Continued)

Table 1 (continued)

Classification	Examples	Mode of action	References
Cytokinins	Indoleacetic Acid, Indole Butyric Acid, and Naphthaleneacetic Acid	Promote cell division, sprouting and root development, seed and fruit development, germination, senescence and response to environmental stresses.	[31]
Abscisic acid	Abscisic acid	Induces embryo ripening, seed dormancy, vegetative growth and processes related to stress tolerance (stomata closure), ripening and fruit abscission	[24]
Ethylene	Ethylene	Diminishes stem and root growth. Helps flower development, fruit ripening, in addition to leaf and flower senescence and fruit abscission.	[26]
Silicates	Salicylic acid	Induces flowering, inhibits ethylene biosynthesis and increases growth. Enables stress regulation and stress response.	[24,26]
Jasmonates	Jasmonic Acid, and Isojasmonic Acid	Helps signalling in plants to respond to biotic and abiotic stress. Promotes seed germination, root growth and flowering.	[24,26]
Brassinosteroids	Brasinolide, Dolicolide and Epibrasinolide	Influence germination, rhizogenesis, flowering, senescence, abscission and ripening processes. They also confer resistance to plants against abiotic and biotic stresses.	[26,32]
Polyamines	Putrescine, Spermidine and Spermine	Are involved in cell division and cell elongation, nucleic acid packaging, DNA replication and rooting.	[27]
Strigolactones	Strigol, Strigyl Acetate and Sorgolactone	Inhibit auxin transport, promote shoot and root growth and plant defence.	[33]
Triazoles	Paclobutrazol	Reduce plant growth, promote flower development and a greater number of leaves and roots, and improve tolerance to environmental or disease stress. Increase the concentration of chlorophyll and antioxidant enzymes such as superoxide dismutase, catalase, ascorbate and peroxidase.	[23,26]

Recent studies report that growth bioregulators, when applied in small concentrations, are easily uptaken and mobilised through the xylem, with the purpose of modifying the physiological behaviour of the plant [4]. There are commercial products that are used as part of the agronomic management of plant growth and development, synthetic analogues of natural bioregulators, including PBZ [25,34].

3 Paclobutrazol (PBZ)

PBZ was first announced in 1986 as a new bioregulator, which was introduced to the market by ICI Agrochemicals (now part of Syngenta) [5]. It is a synthetic compound [(2 RS, 3 RS) -1- (4-chlorophenyl) - 4,4-dimethyl-2- (1 H-1,2,4-triazol-1-yl) -pentan-3 ol] that inhibits vegetative growth [6,8], belonging to the triazole group [9,11]. Chemical properties of PBZ include: molecular weight 293.8, molecular formula $C_{15}H_{20}ClN3O$, melting point 165°C–166°C, density 1.22 g ml^{-1} and water solubility 35 mg L^{-1}. PBZ is a hydrophobic and slightly polar molecule, with hydrophilic parts [35]. It has two chiral centres (two asymmetric carbons), hence the existence of two pairs of enantiomers [(2R, 3R)- and (2S, 3S)-] and [(2S, 3R)- and (2R, 3S)-]. However, among the stereoisomers, 2S and 3S show a higher inhibition efficiency in gibberellin biosynthesis, but 2R and 3R are more easily degraded [36].

PBZ is found as an active ingredient in several commercial products such as: "Cultar® 25 SC" and "Bonzi®" (Syngenta, USA) [5], "Regalis® Plus" (BASF, USA) and "AuStar®" (Chemicals Direct Pty, Ltd., Australia) [16]. It is a non-polar compound with a broad-spectrum nature that is mainly translocated *via* xylem. However, it will depend on the application route, as it can also be transported via phloem [11,37].

The mode of action of PBZ is framed as part of the terpene pathway. This is, it inhibits the biosynthesis of gibberellins by inactivating the enzyme ent-kaurene oxidase, which catalyses their oxidation to ent-kaurenoic acid. This favours the activation of the enzymes geranylgeranyl reductase and phytoene synthase for chlorophyll and abscisic acid biosynthesis, respectively (Fig. 1) [23,38,39]. As a result, it decreases vigour and promotes floral induction and development [9,10,15].

Plant growth and development is associated with cell division and expansion induced by gibberellin activity. PBZ applications inhibit its synthesis; consequently, cell elongation does not occur. In the tree you can see a greater number of leaves, shoots and shorter internodes. Likewise, it increases the thickness of the leaves and reduces the size of stomatal pores [40].

4 Use of PBZ in Fruit Trees

Among the main effects and physiological processes affected that have been reported with the application of PBZ are the synthesis of gibberellins, floral induction, photosynthetic activity by promoting a higher concentration of chlorophyll, reduction of the transpiration rate (closure of stomata) and activation of antioxidant enzymes (superoxide dismutase, catalase, ascorbate peroxidase and peroxidase) related to the mitigation of oxidative stress [23,37]. It is applied *via* foliar and soil at concentrations ranging from 1–200 mg L^{-1} and 0.1–8.0 mg L^{-1}, respectively [39].

The effect of PBZ has been described, for example, in pear "Clapp's" (*Pyrus communis* L.). It was applied *via* foliar at concentrations of 100, 200 and 300 mg L^{-1} during the phenological stage of petal fall, which resulted in a 34% increase in yield [12]. In India walnut "Ullal-3" (*Anacardium occidentale* L.) PBZ was applied to the soil for two years at three concentrations (1, 2 and 3 g L^{-1}), prior to flower opening This resulted in an increase in the number of female flowers and an improvement of 54% and 30% in the concentration of chlorophylls a and b, respectively [9].

In China, PBZ is applied to the soil in November and December to reduce the vegetative growth of "Western Schley" pecan [*Carya illinoinensis* (Wangenh) K. Koch] at concentrations of 30, 90 and 150 mg·cm^2 [14]. Another work on olive (*Olea europaea* L.) "Arbosana" in Tunisia applied PBZ *via* foliar (10 ml L^{-1}) and edaphic (20 ml L^{-1}), where a significant reduction in shoot length and leaf area was found, but the foliar application was more effective. Additionally, the behaviour of yield indicators (number of inflorescences and number of flowers per inflorescence) was modified with respect to the control [41].

Figure 1: Simplified pathway of terpenes involved in gibberellin biosynthesis, and inhibition sites for abscisic acid and chlorophyll formation. Adapted from [23,38,39]

In order to reduce vegetative growth and promote flowering, PBZ applications are made in July at concentrations of 7.5 and 10 g L^{-1}, respectively [42]. In litchi (*Litchi chinensis* Sonn) "China", soil applications of this product (1,2,3 and 4 g L^{-1}) have been made in September, in which an increase in transpiration rate and stomatal conductance was found. Likewise, the dose of 4 g L^{-1} showed the highest yield, vitamin C and anthocyanin content in the fruits [11].

Cruz et al. [43] evaluated the effect of PBZ on lime (*Citrus latifolia* Tanaka) "Tahiti" with a soil application of 800 mg $plant^{-1}$ and found a significant increase in flowering and fruit set. In blackberry

(*Morus alba* L.) "S-146", 5, 10, 25, 50, 100 and 500 mg L^{-1} of PBZ were applied, where an increase in biomass accumulation and the percentage of photochemical efficiency were observed, through the induction of enzymatic antioxidants (superoxide dismutase and catalase) [6].

Authors such as Barman et al. [44] in a research on mango (*Mangifera indica* L.) "Dashehari" applied 3.2 mL m^2 of PBZ in the month of September; they reported a significant increase in flowering and 30% more yield. In another experiment, but with the variety "Namdokmai-sitong", the application was carried out 15 and 45 days after pruning with doses of 500, 1000, 1500 and 2000 mg L^{-1}, which resulted in a decrease in vegetative growth and an improvement in the number of flowers and buds [10]. On the other hand, in Brazil, Do Amaral Brogio et al. [15] carried out foliar applications of PBZ (1750 mg L^{-1}) on avocado (*Persea americana* L.) "Hass" and found that it modified the shape of the fruit and caused a significant increase in fruit size.

Foliar application of 2500 mg L^{-1} PBZ in the flower bud inductive period (20–25 November) promoted flowering by 40% and 51% in orange (*Citrus sinensis* L. Osb.) "Salustiana" and *Citrus x clementina* Hort. Ex tan "Hernandina", respectively [45]. In another study with the orange variety "Navel" grafted on "Carrizo" [*Citrus sinensis* (L.) Osbeck x *Poncirus trifoliata* Raf.], but with the soil application of 1000 mg L^{-1} prior to harvest, a significant improvement was obtained in the colour (color tonality) and carotenoid content of the harvested fruits with respect to the control [46].

As in other countries, PBZ is used in Mexico to promote flowering in mango (*Mangifera indica* L.) "Ataulfo" as reported by Pérez-Barraza et al. [19]. These authors applied PBZ 30 days after pruning and observed an earlier entry into the flowering period (45 days) with respect to the control. In the "Manila" variety, the same authors when applying 10 mL of PBZ per tree, found a significant variation by bringing forward flowering by 15 days, and increasing fruit weight and size [18].

In guava (*Psidium guajaba* L.) "Calvillo" it was applied at concentrations of 1 mL $tree^{-1}$ and 2 mL $tree^{-1}$ at the base of the stem after pruning. This affected vegetative growth with a reduction of between 15% and 41%, and an increase in fruit number of 30% [16]. The dose, effect and type of application (edaphic and foliar) of PBZ on various fruit trees are described in Tab. 2.

Table 2: Doses and effects of PBZ applied to various fruit trees

Fruit-tree	Form of application	Dose	Effect	Source
Anacardium occidentale L.	Edaphic	300	Reduced vegetative growth. Flowering increased by 30% Increased female flower production by 45% to 54%. Increased chlorophyll a and b concentrations by 54% and 30%, respectively.	[9]
Litchi chinenesis Sonn	Edaphic	100 200 300 400	Increase the concentrations of chlorophyll a and b. Reduced leaf nitrogen content (1.5%). The high dose increased yield by 60%.	[11]
*Carya illinoinensis (*Wangenh) K. Koch	Edaphic	300 900 1500	Annual delay in terminal shoot growth relative to the control. Increased short shoot production by 60%, with a reduction in long shoots for all doses relative to the control. Increased flowering by 35% at the lower dose rate.	[14]
		1250	Inhibited vegetative growth (reduced shoot length).	[13]

(Continued)

Table 2 (continued)

Fruit-tree	Form of application	Dose	Effect	Source
Mangifera indica L.	Edaphic	750 1000	Reduced shoot growth by 30% and 13%, respectively. Modified flowering date by an average of 45 days compared to the control. Both doses increased the number of panicles. Significantly increased yield. Increased yield eight times more than the control at both doses. Improved fruit quality at both doses.	[42]
Mangifera indica L.	Edaphic	700 1000 1300 1600 1900	The lower doses increased gas exchange by 23% compared to the higher doses. Increasing doses of paclobutrazol resulted in a 31% decrease in sugars 31% decrease in total soluble sugars, 54% decrease in reducing sugars and 29% decrease in non-reducing sugars.	[47]
	Foliar	320 2500 1000	25% increase in flowering. 60% increase in flowering and bud differentiation. 80% increased flowering and significantly decreased shoot length.	[44]
Olea europaea L.	Edaphic	10 20	Application inhibited growth by 55% and 82% for each dose.	[41]
Morus alba L.	Edaphic	5 10 25 50 100 500	Increased biomass accumulation (leaves) and net photosynthetic rate (38%).	[6]
Psidium guajava L.	Edaphic	100 200	Growth rate decreased by 15% to 41% compared to the control. Increase of 30% and 37% in yield and number of fruits, respectively.	[16]
Persea americana Mill.	Foliar	1750	Increased fruit length, diameter and weight by 11%, 5.3% and 1.5%, respectively, with respect to the control.	[15]
Pyrus communis L.	Foliar	200	Inhibited vegetative growth. Increased fruit quality (total soluble solids, total sugars and juice) and yield.	[12]
Vitis vinifera L.	Foliar	200 300	Increased bunch weight by 54% (107.41 g) compared to the control (69.84 g) as well as an increase in yield by 64%. Increased chlorophyll content by 31.5% and reduced shoot length by 33.8%.	[48]
Myrica rubra Sieb. et Zucc.	Foliar	100 200	Increased fruit weight by 50%. Increased fruit weight by 30.5%, increased soluble solids concentrations by 13% and decreased total acidity by 38%.	[49]

Note: The dose is expressed in milligrams per litter ($mg\ L^{-1}$).

PBZ can be a tool to increase yields in fruit crops such as pear, litchi, mango, if it is applied in adequate concentrations and at the appropriate phenological stages. However, it is necessary to know the physiological processes of the species which are implied in the final response, and what is intended to be modified. This is because it can have an impact on improving the yield and quality of the harvested product (Fig. 2) [23,39,41].

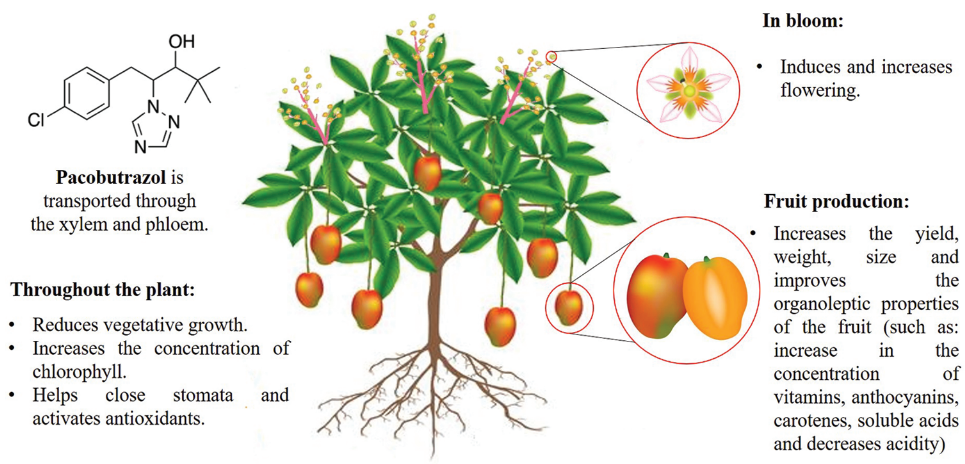

Figure 2: Summary of main key mechanisms that paclobutrazol targets in fruit trees

5 Effects of PBZ on the Environment

Recent studies have demonstrated that the residual nature of PBZ affects soil microbial life (reduction in the number of fungi, bacteria, actinomycetes and earthworms), which impairs plant growth and development [21]. In addition, leaching and contamination of aquifers can occur [8,17]. On the other hand, the behaviour of PBZ in soil has a residual effect of 450 to 950 days and can be maintained at a depth of 60 to 120 cm [37]. Other authors reported that PBZ has an easy uptake by the crop roots, and can have a persistence in soil from 43 to 618 days, with concentrations ranging from 1.1 to 50 mg kg^{-1}. Another important effect is the inhibition of root growth and development of plantations in soils with previous PBZ applications [35]. On the other hand, in mango, Lin et al. [50] report a reduction in the density of beneficial soil microorganisms, in particular the number of bacteria, fungi and actinomycetes by 58%, 28% and 28%, respectively, when evaluating PBZ applications.

The life of PBZ in water is shorter (i.e., no longer than 3 weeks), but it can cause potential damage to the flora and fauna [37]. The presence of PBZ in water can cause various physiological effects in fish (survival rate, embryonic hatching, failure in the development of the skeleton of the head and eyes) [21,51]. Such is the case of *Daphnia magna* (water flea) which showed physical deformities when exposed to 240 μg L^{-1} of PBZ in the embryonic stage [39]. When evaluating the survival and deleterious effect of PBZ (0.34, 3,4, 17 μm) applied to zebrafish (*Danio rerio*) embryos at 24, 36, 48, 60, 72 and 96 hours after fertilisation, Wang et al. [21] report the development of pericardial oedema, cardiovascular dysfunction and skeletal malformations. Osuna-García et al. [17] found traces of PBZ in fruit samples when applying doses of 5 and 2.5 mL of PBZ per tree to "Tommy Atkins" mango; however, the authors indicate that these concentrations are not harmful to human health. In some countries it is prohibited and/or restricted the use of PBZ due to the residual effects on fruits that can be harmful to human health (Tab. 3) [35].

PBZ residues can affect human health through direct contact, inhalation, contamination of water bodies, consumption of contaminated fish and fruits containing residues [37]. Adverse health effects can occur after years of minimal exposure in the environment, food and water [52]. There are few studies on the harmful effects caused to human health by ingestion or contact with PBZ (Fig. 3) [53]. Experiments have been carried out on pregnant rats were fed with doses of 1.0 mg kg^{-1} PBZ and the results have been extrapolated to humans: no reproductive effects were observed, but malformations (cleft palate) were determined. These malformations were attributed to a toxic effect of PBZ. Further studies were recommended to determine whether PBZ is the cause of this effect [54,55].

Table 3: Maximum residue limit in fruits for paclobutrazol in some countries

Countries	China	Japan	European Union	South Korea	Australia	USA	Sweden
LMR (mg kg^{-1})	0.5	0.01	0.01	0.01	0.01	Ornamental	Forbidden

Note: LMR–maximum residue limit.

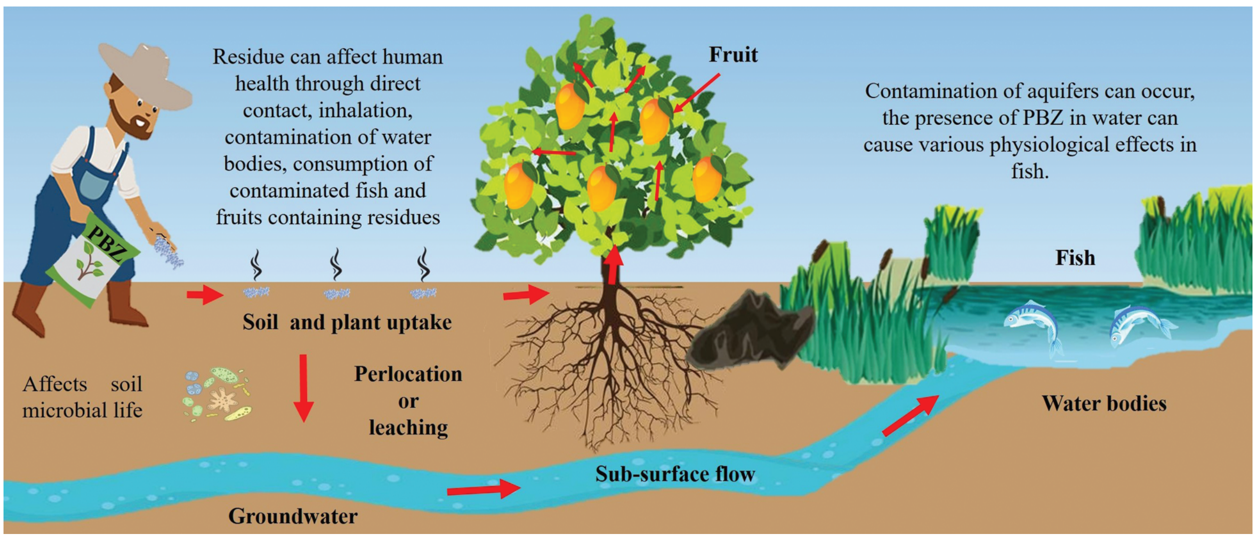

Figure 3: Effect of paclobutrazol on living beings

6 Future Perspectives

Paclobutrazol is a growth inhibitor and also belongs to the triazol group. The use of this product on fruit trees (mango, lime, apple and guava) inhibits the biosynthesis of gibberellins; cell division occurs, cell elongation and expansion do not occur. This allows a greater production of shoots, number of leaves and internodes, but they will be shorter. PBZ induces flowering with a consequent increase in fruit yield, weight, size, and it improves the organoleptic properties of fruit.

On the other hand, in some countries it is prohibited and/or restricted by the documentary evidence of its residual and harmful effects on the environment (soil and groundwater) and human health (LMR in fruits). However, fruit production is associated with an extensive use of PBZ in Latin America. There is little evidence of a legal framework that allows users to implement the optimal use of this product, to mitigate possible effects on the environment and human health. For this reason, the agronomic management of PBZ must have protocols that seek its regulation with a rational and sustainable approach.

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