AF-773
[1-6]

RESEARCH ARTICLE Indian Journal of Agricultural Research,

Paclobutrazol Biodegradation of Native Bacteria in Mango Orchards in Mekong Delta, Vietnam

D.T.T. Pham[1], T.M. Bui[1], H.D.N. Thai[1], H.T.T. Vo[2], D.T. Vo[1], L.M.T. Vu[1]

10.18805/IJARe.AF-773

ABSTRACT

Background: Paclobutrazol (PBZ), a plant growth regulator, is usually used to promote off-season flowering in mango orchards in the Mekong Delta, Vietnam. Several studies demonstrated that PBZ degradation in the soil takes several months and its residue effects to the soil environment and consequently to the plants and microorganism community. The objective of this study was to identify native bacteria that can biodegrade PBZ in the soil of mango orchards.

Methods: Soil samples were collected in thirty mango orchards in Vietnam's Mekong Delta. One gram of soil was mixed with distilled water and injected into a PBZ culture medium. After culturing, bacterial colonies were isolated and transferred onto the TSA medium in order to characterize morphological and biochemical assays and to evaluate PBZ biodegradation ability. Four strains of bacteria, which had high PBZ biodegradation, were identified and evaluated PBZ biodegradation ability. Additionally, 16S rDNA region was sequenced.

Result: The results of identification indicated that the four high PBZ biodegradation bacterial strains related to *Pseudomonas fluorescens, Pseudomonas nitroreducens, Burkholderia cepacia, Acinetobacter seifertii.* After 30 days, the content of paclobutrazol biodegradable by these bacteria was 36.51%, 36.23%, 34.19% and 33.56%, respectively, at an initial concentration of 60 ppm.

Key words: Acinetobacter, Biodegradation, Burkholderia, Mango, Mekong delta, Paclobutrazol, Pseudomonas.

INTRODUCTION

Paclobutrazol (PBZ) is a plant growth retardant that affects to growth and development of plants based on changing concentration of important hormone of plants (Desta and Amare, 2021). PBZ was applied for several crop plants at suitable doses to reduce the plant height, control lodging and promoted off-season flowering, as well as to improve fruit yield and quality (Kishore el al., 2015; Dewi *et al*. 2016; Mabvongwe *et al*., 2016). However, when irrigated directly to the soil with high concentration, PBZ has the ability to accumulate in the soil for several years, reducing crop growth in the next crop (Santos *et al*., 2014). This substance has the potential to cause mild skin and eye irritation, but PBZ is not an animal sensitizer (Kumar *et al*., 2021).

Mango is a major fruit crop in Vietnam that is responsible for an important part of fruit export of the country. In Vietnam, mango trees naturally flower annually in November and January and the fruits ripen from February to May, but low market prices. In order to gain the higher benefit, the farmers tend to induce off-season flowering by the use of PBZ.

Farmers also tend to apply at higher doses than recommended and re-apply annually therefore an accumulation of PBZ in the soil is alarmed.

Application of microbial cleaning technology for removal of agrichemicals is an effective and inexpensive way to treat pollution in agricultural soil (Li *et al*., 2009; Kanissery and Sims, 2011; Polthanee *et al*., 2019; Gurjar and Hamde, 2021). Some studies shown that bacteria belonging to the genus *Pseudomonas* have the ability to biodegrade toxins in the soil environment (Seeger *et al*., 2010; Chawla *et al*., 2013; Bano *et al*., 2018). However, the number of related studies is still limited. The current study aimed to screen promising bacterial strains and evaluate their PBZ biodegradability ability.

[1]Faculty of Agronomy and Nong Lam University, Ho Chi Minh City, Vietnam.
[2]Faculty of Bio-Sciences, Nong Lam University, Ho Chi Minh City, Vietnam.

Corresponding Author: D.T.T. Pham, Faculty of Agronomy and Nong Lam University, Ho Chi Minh City, Vietnam.
Email: pttduong@hcmuaf.edu.vn

How to cite this article: Pham, D.T.T., Bui, T.M., Thai, H.D.N., Vo, H.T.T., Vo, D.T. and Vu, L.M.T. (2023). Paclobutrazol Biodegradation of Native Bacteria in Mango Orchards in Mekong Delta, Vietnam. Indian Journal of Agricultural Research. doi: 10.18805/IJARe.AF-773.

Submitted: 12-12-2022 **Accepted:** 20-03-2023 **Online:** 06-04-2023

MATERIALS AND METHODS

The study was carried out from November 2020 to June 2022 at the laborary of Agronomy Faculty, Nong Lam University, Ho Chi Minh City, Vietnam.

Soil sampling

Soil samples were collected from 30 mango orchards with a PBZ usage history of over 5 years. The soil was sampled 6 months after the newest PBZ treatment. The soil was collected at a depth of 0-20 cm. Samples collected in the same garden were mixed to form a uniform sample.

Bacterial isolation

Medium preparation: PBZ (Sigma Aldrich USA) and other chemicals purchased from Merck Ltd. The mineral medium contained 1.0 g NaCl/l; 0.5 g NH_4Cl/l; 0.2 g $MgCl_2.6H_2O$/l; 0.02 g $CaCl_2.2H_2O$/l; 0.5 g K_2HPO_4/l, 0.5 g KH_2PO_4/l (Chen *et al*., 2010) supplemented with 15.31 mg PBZ/l. The pH was adjusted to 7.0 and then autoclaved at 121 degrees Celsius for 20 minutes.

One gram of soil and 25 mL of mineral medium were gently mixed and shaken on a horizontal shaker at 90 rpm at room temperature without illumination for 15 days. After 15 days of culture, 1 mL of the first-generation bacterial solution was poured into a sterilized 100 mL Erlenmeyer flask containing 24 mL of mineral medium solution supplemented with 15 ppm PBZ. The bacterial solution was then continued to culture for another 15 days. The entire procedure was repeated continuously for three times. For the final culture, solutions sucked out 1 mL of the solution and diluted the sample to a concentration of 10^{-5}. After the solutions were sucked out 10 µL of the above sample solution at concentrations of 10^{-5} to spread on TSA medium in a petri dish. After 72 hours, bacterial colonies appearing on agar plates were counted and morphologically and biochemically distinguished.

Evaluation of the PBZ biodegradability of bacteria

Ninety bacterial strains were selected and enriched in a Erlenmeyer flask that contained 50 mL of the GYM nutrient solution (dissolve 10 g of glucose and 10 g of yeast extract in 1000 mL of distilled water) after 72 hours then shaken at 90 rpm in the dark. The biomass of the bacterial strains was obtained by transferring the entire GYM nutrient solution containing the bacterial biomass into a sterilized 50 mL falcon tube. The process of centrifugation was repeated continuously for three times to completely remove the residual carbon source from the GYM medium.

A solution containing 15 ppm of paclobutrazol (PBZ) was prepared by adding 400 µL of bacterial solution, 3.6 mL of mineral medium, 15 ppm of PBZ and 1 mL of distilled water to a tube. The tubes were placed in the dark on a shaker at laboratory temperature and agitated at 90 rpm. After 15 days and 30 days of culture, the residual PBZ concentration was extracted for quantitation by a High-Performance Liquid Chromatography (HPLC) using a C18 column (length: 25 cm, inner diameter: 4.6 mm), the ratio of acetone nitrile to demineralized water: 50-50 and wavelength 225 nm.

Four bacterial strains with the highest PBZ biodegradation ability were assayed on medium supplemented with PBZ at concentrations of 15, 30, 45 and 60 ppm for 15 and 30 days then were identified using molecular biology-based techniques.

Identification of bacteria species by molecular biology

Four bacterial strains were identified by sequencing the 16S rDNA. Purified DNA was amplified with the primer pair: 27F-1495R using temperature regime of 94°C (3 minutes), 25 cycles: 92°C (30 seconds) - 50°C (45 seconds) - 72°C (30 seconds), 72°C (4 minutes).

The amplification quality was checked by the use of agarose gel electrophoresis before sequencing. The sequencing results were aligned with other sequences on the genebank (http://blast.ncbi.nlm.nih.gov/Blast.cgi) to estimate the similarity and species speculation.

RESULTS AND DISCUSSION

Morphological identification of bacterial strains

Colonies of the bacterial strains when cultured on TSA medium exhibited different color, shape, texture. The results of Table 1 showed that the bacteria with coccus cell shape was dominant and accounted for 62.2%, while that of rod cells was only 37.8%. The colonies isolated were mainly white (47.8%), milky white (50%) but some were in yellow (2.2%) on TSA medium. Most of the colonies were round in shape (92.2%), with convex elevation (93.3%), slimy and moist texture (85.6%) and regular entire margins (56.7%).

Biochemical tests identification of the bacterial strains

ll bacterial strains were examined through eleven biochemical test reactions. The results of Table 2 showed that gram-negative bacteria accounted for 60%, while gram-positive bacteria were only (40.0%). The ratio between non-motility and motility bacteria had no significant difference, 55.6% and 44.4%, respectively. Most of the bacteria were positive in biochemical tests with Catalase (94.4%), Oxidase (84.4%) enzyme activities. A dominant number was also positive in Citrate utilization (95.6%), H_2S production (73.3%) and Methyl red reaction (87.8%). For the Indole production test, the majority of bacterial strains had negative results

Table 1: Characteristics of cell and colony morphology of the isolated bacterial strains.

Characters	Ratio (%)
Cell shape	
Rod	37.8
Coccus	62.2
Colony shape	
Round	92.2
Irregular	7.8
Colony color	
White	47.8
Milky white	50.0
Yellow	2.2
Colony elevation	
Convex	93.3
Raised	5.6
Flat	1.1
Colony texture	
Slimy, moist	85.6
Dry, mucous	14.4
Colony margin	
Entire	56.7
Lobate	16.7
Undulate	26.7

(71.1%). The results of biochemical tests suggested that the bacterial community in PBZ treated mango orchards was diverse. The research by Bano *et al*. (2018) identified the bacterial species belonging to the genera *Bacillus*, *Enterobacter*, *Pseudomonas*, *Proteus* and *Serratia* and *Lactobacilli* genera existed mainly in the mango growing soil in Sindh, Pakistan.

Evaluation of PBZ biodegradability using ninety bacterial strains

All bacterial strains were cultured in mineral medium supplemented with PBZ at a concentration of 15 ppm. The results showed that several bacterial strains showed PBZ biodegradable ability after 15 and 30 days of culture. At 15 days after culture, numbers of bacterial strains capable of degrading PBZ in different PBZ treated concentrations had significantly different and ranged from 14.70 to 32.70%. Most of the bacterial strains showed the ability to biodegrade PBZ in range from 16.81 to 19.90% and included 41 strains that accounted for 45.5%. The strains shown ability to biodegrade PBZ in range from 19.91 to 22.90% including 34 strains that accounted for 37.8%. There were four bacterial strains capable of degrading PBZ in a range from 26.01 to 32.70% (Fig 1).

At 30 days after culture, the initial PBZ concentration decreased from 20.40% to 39.60%. There are 48 out of 90 bacterial strains (accounting for 53.30%) that had the ability to reduce between 22.71% to 25.80% of the initial PBZ concentration. The number of bacterial strains capable of reducing 25.81%% to 28.80% of the initial PBZ concentration was 31 strains (accounting for 34.4%). There were only 6 strains of bacteria capable of degrading PBZ from 28.81% to 39.60%. The number of bacterial strains that had PBZ biodegradation ability from 31.81 to 39.60% were four strains (Fig 2).

In the experiment conditions, PBZ was the sole source of carbon for the growth of bacteria. Depending on the adaptability and the need to use carbon and the mechanism of PBZ biodegradation, bacterial strains had different PBZ-biodegradation abilities. Chen *et al*. (2010) indicated that *Pseudomonas* sp. isolated from industrially contaminated soil in China was able to biodegrade up to 60% of PBZ at an initial concentration of 54 ppm in 48 hours and 98% of PBZ at a concentration of 3.4 ppm in 36 hours. According to research by Dang *et al*. (2014), *Burkherderia* sp. was isolated on mango soil in Mekong Delta, Vietnam showed the ability to biodegrade 15.53% to 16.41% of the initial 15 ppm PBZ concentration after 15 days of culture. The study by Santos *et al*. (2014) showed that bacteria of the genus *Pseudomonas* were able to biodegrade PBZ from 8 to 95% of the initial concentration of PBZ in a period of 40 days.

Table 2: Results of biochemical tests of bacterial strains.

Characters	Ratio (%)	
	Positive result (+)	Negative result (-)
Gram stain	40.0	60.0
Motility	44.4	55.6
Catalase	94.4	5.6
Oxidase	84.4	15.6
Glucose fermentation	41.1	58.9
Vogas Praskaur	65.6	34.4
Citrate utilization	95.6	4.4
Indole production	28.9	71.1
H_2S production	73.3	26.7
Nitrate reduction	67.8	32.2
Methyl red	87.8	12.2

Determination of PBZ biodegradability of the selected bacterial strains at different concentrations and times

The four most prominent PBZ biodegradable bacterial strains selected in the experiment as the above were determined PBZ biodegradability at different concentrations, specifically 15, 30, 45 and 60 ppm. At different PBZ concentrations, the concentration of biodegradable PBZ at 30 days after culture was higher than at 15 days after culture. However, the rate of PBZ biodegradation was rapid in the first 15 days and gradually decreased during the next 15 days.

In the mineral medium containing PBZ at a concentration of 15 ppm, the PBZ biodegradation ability of the four bacterial strains was not significantly different among the four strains but all showed clearly higher than that of the control sample (Fig 3A). At 15 days after culture, the PBZ concentration decreased from 28.13% to 33.62%. At 30 days after culture, the PBZ biodegradation efficiency was from 37.11% to 41.89%. When the concentration of PBZ in the

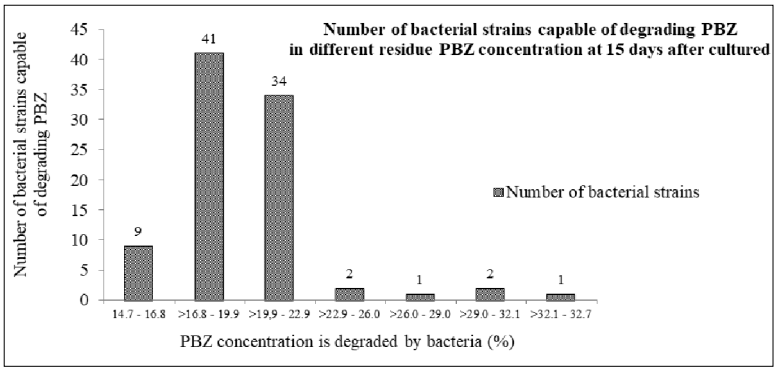

Fig 1: Number of bacterial strains capable of biodegrading PBZ in different residue PBZ concentrations at 15 days after culture.

medium increased to 30 ppm, the rate of PBZ biodegradation tended to slow down. However, the biodegradation efficiency of PBZ among the four bacterial strains was not significantly different and was higher than that of the control sample.

The difference in PBZ biodegradation at an initial PBZ concentration of 45 ppm was not significant as compared to the initial concentration of 30 ppm. At 15 and 30 days after culture, the concentrations of biodegraded PBZ were 23.42% to 28.52% and from 34.7% to 39.88%, respectively (Fig 3C). At an initial PBZ concentration of 45 ppm, the difference in PBZ biodegradability of the four bacterial strains was not significant, but higher than that of the control. When enhancing the concentration of PBZ in the culture medium up to 60 ppm, it was shown that at 15 days after culture, the difference in PBZ biodegradation ability in the four bacterial strains was different from each other and clearly higher than the control. At 30 days after culture, the PBZ biodegradation among the four bacterial strains reached an equilibrium, the difference was not statistically significant but clearly higher than that of the control. At this time, the concentration of biodegraded PBZ ranged from 33.56% to 36.51% (Fig 3D).

When enhancing the initial concentration of PBZ in the culture medium, the concentration of degraded PBZ tended to be decreased. This result is consistent with the study of Vaz *et al*. (2015) that showed the higher residual PBZ concentration resulted in a lower biodegradation efficiency. PBZ residue at high concentrations in the medium inhibited the metabolism of microorganisms.

Identification of four bacterial strains by morphology, biochemistry test and molecular biology

Colony and cell morphology and biochemical tests are characteristic features used for the preliminary identification of bacteria. These characteristics of four bacterial strains with high PBZ-biodegradation ability under laboratory conditions were presented in Table 3.

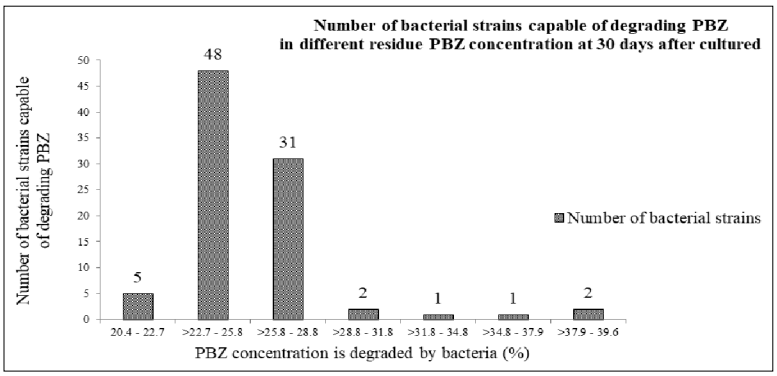

Fig 2: Number of bacterial strains capable of biodegrading PBZ in different residue PBZ concentrations at 30 days after culture.

Table 3: Colony and cell morphology and biochemical test results of four selected bacterial strains.

Characters	Bacterial strains			
	NL-P5	NL-P7	NLP-12	NL-P17
Colony color	White	Creamy-white	White	Creamy-white
Colony shape	Round	Round	Round	Round
Colony elevation	Convex	Convex	Convex	Convex
Colony margin	Entire	Entire	Entire	Entire
Cell shape	Coccus	Rod	Rod	Rod
Gram stain	-	-	-	-
Motility	-	+	+	+
Catalase	+	+	+	+
Oxidase	-	+	+	+
Glucose fermentation	-	+	+	-
Vogas Praskaur	-	-	-	+
Citrate utilization	+	+	+	-
Indole production	-	-	-	-
H_2S production	-	-	-	+
Nitrate reduction	-	+	-	-
Methyl red	-	-	+	+

Note: Positive result (+), negative result (-).

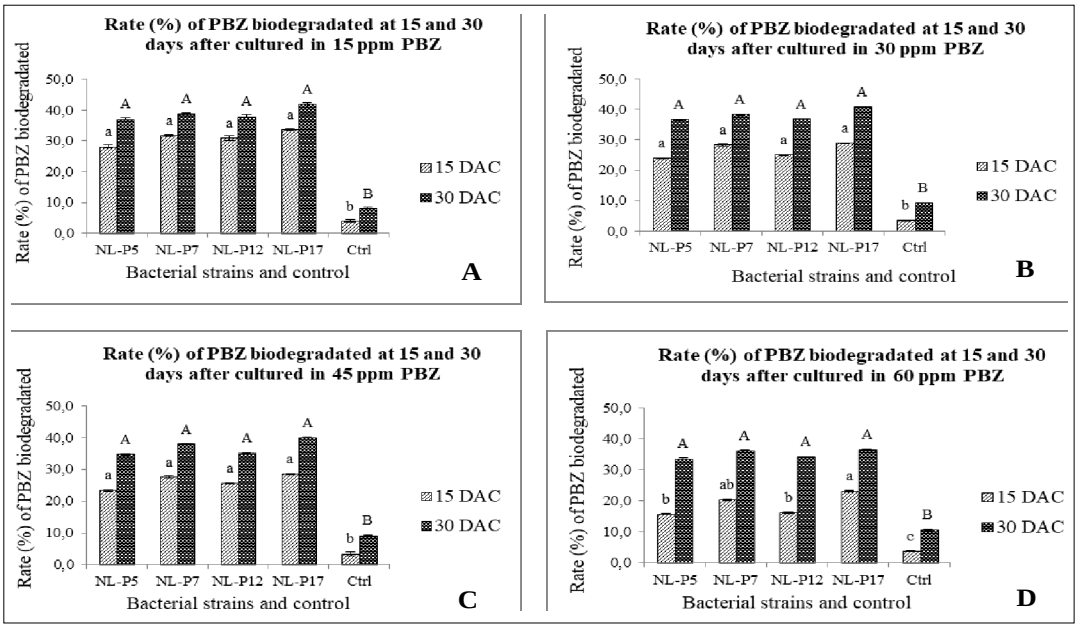

Fig 3: Biodegraded rate (%) of PBZ at 15 and 30 days after culture on media supplimented with PBZ 15 (A), 30 (B), 45 (C), or 60 (D) ppm.

The results showed that the morphological characteristics of the colonies on TSA medium had similarities between the four isolates. The colonies of the four selected strains of bacteria were all white (NL-P5 and NL-P12) or milky white (NL-P7 and NL-P17), round shape, with convex elevation and entire margin. There were three strains: NL-P7, NL-P12 and NL-P17 had rod cells, while NL-P5 strain had coccus cells. All the four strains were gram-negative. Research by Vaz *et al*. (2015) also showed that, among bacterial strains that were resistant to PBZ, 89% belonged to gram-negative bacteria. These four strains all reacted positively to the Catalase but negatively to the Indole production test. There were significant differences in the results of the biochemical test for Motility, Oxidase, Glucose fermentation, Vogas Praskaur, Citrate utilization, H_2S production, Nitrate reduction and Methyl red test (Table 3).

The identification of the four strains based on sequencing of the 16S rDNA using 27F and 1495R primer pair showed that the bacterial strains NL-P5, NL-P7, NL-P12 and NL-P17 related to *Acinetobacter seifertii, Pseudomonas nitroreducens, Burkholderia cepacia* and *Pseudomonas fluorescens*, respectively. This result was consistent with the study of Chen *et al*. (2010), Santos *et al*. (2014), Dang *et al*. (2014) and Vaz *et al*. (2015).

CONCLUSION

Four bacterial strains NL-P5, NL-P7, NL-P12 and NL-P17 exhibited the highest PBZ biodegradation ability. When testing the ability of these four bacterial strains to biodegrade PBZ at different concentrations of PBZ, it indicated that PBZ decomposition activity of the four strains decreased at the higher PBZ concentration. Four bacterial strains were identified through 16S rDNA sequencing indicated that they were closely related to *Pseudomonas fluorescens, Pseudomonas nitroreducens, Burkholderia cepacia and Acinetobacter seifertii*. The concentrations of PBZ in the minimum mineral medium with biodegradation were 36.51%, 36.23%, 34.19% and 33.56%, respectively, for 30 days after culture at an initial PBZ concentration of 60 ppm. There is a need to further evaluate the impact of the environmental factors on the efficiency of the selected bacterial strains in PBZ biodegradation in field conditions.

ACKNOWLEDGEMENT

We would like to express our sincere thanks to the support of the NLU in HCMC and the Ministry of Education and Training of Vietnam (Project number: B2021-NLS-02).

Conflict of interest: None.

REFERENCES

Bano, S., Tunio, S.A., Bugti, H., Khan, S. and Seeger, A.N.J. (2018). Exploring the rhizospheric bacterial communities of *Mangifera indica*. B. Life and Environmental Sciences. 55(2): 65-70.

Chawla, N., Suneja, S. and Kukreja, K. (2013). Isolation and characterization of chlorpyriphos degrading bacteria. Indian Journal of Agricutural Research. 47(5): 381-391.

Chen, J., Xu, L., Giesy, J.P. and Jin, H.J. (2010). Biodegradation of paclobutrazol by a microbial consortium isolated from industrially contaminated sediment. Toxicological and Environmental Chemistry. 92: 8, 1487-1494.DOI:10.1080/02772241003689868.

Dang, P.T.T., Do, T.X., Duong, M.V. and Nguyen, K.N. (2014). Isolation of native bacterial strains having a degradation capacity for PBZ originate from orchard gardens in some provinces of Mekong Delta, Vietnam. Journal of Science, Can Tho University. 32: 82-86.

Desta, B. and Amare, G. (2021). Paclobutrazol as a plant growth regulator. Chemical and Biological Technologies in Agriculture. 8(1): 1-15.

Dewi, K., Agustina, R.Z. and Nurmalika, F. (2016). Effects of blue light and paclobutrazol on seed germination, vegetative growth and yield of black rice (*Oryza sativa* L. 'Cempo Ireng'). Biotropia. 23(2): 84-95.

Gurjar, M.M. and Hamde, V.S. (2021). Degradation of pyrethroid insecticide- cypermethrin (25% ec) using soil microorganisms and detection of degradation products. Indian Journal of Agricultural Research. 55(5): 563-569.

Kanissery, R.G. and Sims, G.K. (2011). Biostimulation for the enhanced degradation of herbicides in soil. Applied and Environmental Soil Science. 1-10.

Kishore, K., Singh, H.S. and Kurian, R.M. (2015). Paclobutrazol use in perennial fruit crops and its residual effects: A review. Indian Journal of Agricultural Sciences. 85(7): 863-72.

Kumar, G., Lal, S., Bhatt, P., Ram, R.A, Bhattacherjee, A.K., Dikshit, A. and Rajan, S. (2021). Mechanisms and kinetics for the degradation of paclobutrazol and biocontrol action of a novel Pseudomonas putida strain T7. Pesticide Biochemistry and Physiology. 175: 104846. doi:10.1016/j.pestbp.2021.104846.

Li, X.J., Lin, X., Li, P.J., Liu, W., Wang, L., Ma, F. and Chukwuka, K.S. (2009). Biodegradation of the low concentration of polycyclic aromatic hydrocarbons in soil by microbial consortium during incubation. Journal of Hazardous Materials. 172: 601-605.

Mabvongwe, O., Manenji, B.T., Gwazane, M. and Chandiposha, M. (2016). The effect of paclobutrazol application time and variety on growth, yield and quality of potato (*Solanum tuberosum* L.). Advances in Agriculture. 7:1-5.

Polthanee, A., Manuta, P. and Sirikan, J. (2019). Effect of paclobutrazol application time on growth, yield and starch content of two cassava cultivars under rainfed conditions of Northeastern, Thailand. Indian Journal of Agricutural Research. 53(6): 723-727.

Santos, E., Vaz, F. and Gouvei, E. (2014). Increase in biodegradation of paclobutrazol in soils. BMC Proceedings, 8 (Supply 4), P203.

Seeger, M., Hernández, M., Méndez, V., Ponce, B., Córdova, M. and González, M. (2010). Bacterial degradation and bioremediation of chlorinated herbicides and biphenyls. Journal of Soil Science Plant Nutrition. 10(3): 320-332.

Vaz, F., Santos E., Silva, S., Araujo, A., Stamford, T., Bandeira, A., Brasileiro, A.C., Stamford, N.P., Mouco, M.A. and Gouveia, E. (2015). Biodegradation of paclobutrazol - A plant growth regulator used in irrigated mango orchard soil. New Advances and Technology. 5: 85-105.