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Gibberellic Acid and Tween 20 Increases Napier Grass Tolerance to Synthetic Pyrethroid

Khanitta Somtrakoon[1], Wilailuck Khompun[2], Chonlada Dechakiatkrai Theerakarunwong[2] and Waraporn Chouychai[2]*

[1]*Department of Biology, Faculty of Science, Mahasarakham University, Mahasarakham 44150, Thailand*
[2]*Department of Science, Faculty of Science and Technology, Nakhonsawan Rajabhat University, Nakhonsawan 60000, Thailand*

ABSTRACT

The wide use of synthetic pyrethroids has increased their contamination in agricultural soil, so removing this pollutant from agricultural sites is necessary. Phytoremediation offers promise for agricultural soil decontamination as it is an environmentally friendly and green method. In this study, Napier grass cv. Pakchong 1 (*Pennisetum purpureum* x *Pennisetum Americanum*), cuttings with or without soaking in gibberellic acid (GA_3) with and without the surfactant Tween 20, were planted in synthetic pyrethroid contaminated soil for 20 days. The results showed that the synthetic pyrethroid reduced shoot and root growth, reduced the pigment content and increased the proline content in the leaves of Napier grass cv. Pakchong 1, and GA_3 soaking alone was the most appropriate method to alleviate synthetic pyrethroid phytotoxicity. However, planting with Napier grass cv. Pakchong 1 did not enhance soil biodegradation of cypermethrin, deltamethrin, permethrin, and fenvalerate. Napier grass did not accumulate synthetic pyrethroids within the shoot and root tissue, as the bioconcentration factor for each compound was below 1. Indigenous soil microorganisms caused a decrease in these synthetic pyrethroids. Napier grass could tolerate and grow well in pyrethroid-contaminated soil, and a method to enhance the plant's capacity to remove pyrethroid from the soil should be developed.

Keywords: Cypermethrin, deltamethrin, gibberellin, Napier grass, Tween 20

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E-mail addresses:
khanitta.s@msu.ac.th (Khanitta Somtrakoon)
wililuck.k@nsru.ac.th (Wilailuck Khompun)
chonlada.d@nsru.ac.th (Chonlada Dechakiatkrai Theerakarunwong)
waraporn.c@nsru.ac.th (Waraporn Chouychai)
*Corresponding author

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INTRODUCTION

Agricultural and public health use of insecticides worldwide has increased environmental contamination. Synthetic pyrethroids are short-lived in the environment and have low acute toxicity to mammals when compared with organochlorine, organophosphorus, and carbamate pesticides, but it is highly toxic to fish and bees (Thatheyus & Selvam, 2013). Synthetic pyrethroids were synthesised as analogues and derivatives of pyrethrin. This compound was an analogue of pyrethrum, a natural compound extracted from *Chrysanthemum cinerariaefolium* and *Chrysanthemum cineum*, which have been used as natural insecticides for a long time. However, synthetic pyrethroids were more toxic and environmentally recalcitrant than natural pyrethrum. There are many synthetic pyrethroids widely used in Thailand, including type I pyrethroids (without a cyano group), such as permethrin and type II pyrethroids (with a cyano group), such as cypermethrin, fenvalerate, and deltamethrin (Thatheyus & Selvam, 2013). However, the high use in agriculture of pyrethroid pesticides can cause environmental contamination in agricultural areas and should be a concern.

There have been many reports of synthetic pyrethroid contamination. Cypermethrin was reported to show a high frequency of contamination in agricultural soil in Jordan (Kailani et al., 2021). Deltamethrin was reported to contaminate agricultural soil in northern Portugal as 15.7–101.7 ng/g soil during the summer (Bragança et al., 2019). Permethrin and deltamethrin are pyrethroid pesticides reported contaminating tea plantation areas in the Citarum watershed, Indonesia. Permethrin was found at 0.18–0.28 μg/g soil in the wet season and 0.16–0.32 μg/g soil in the dry season, while deltamethrin was found only in the wet season at a concentration of 0.09-0.12 μg/g soil (Ariyani et al., 2020). The soil concentration of synthetic pyrethroid in Thailand has been rarely found, but cypermethrin has been reported as the main insecticide used in rice farms in Ang Thong and Pranakhon Si Ayutthaya Provinces, central Thailand (Maneepitak & Cochard, 2014). This contamination increases the chance of human exposure to this pesticide, and phytoremediation of pyrethroid-contaminated soil in agricultural areas will be an appropriate method for environmental remediation.

The phytoremediation of synthetic pyrethroid contamination in soil, such as cypermethrin and deltamethrin, is still limited (Aioub et al., 2019), while some aquatic plants (*Eichornia crassipes* and *Pistia strateotes*) and algae (*Chaetomorpha sutoria*, *Sirogonium sticticum*, and *Zygnema* sp.) could remove 58–76% of the pyrethroid contamination in water within seven days (Riaz et al., 2017). However, phytoremediation is safe and environmentally friendly for synthetic pyrethroid removal from agricultural soil. The low water solubility and high log K_{ow} of cypermethrin limit the plant uptake and accumulation in shoot biomass (Aioub et al., 2019; Zhu & Zhang, 2008). A possible mechanism for pyrethroid phytoremediation could be phytostimulation of rhizospheric bacteria activity via root exudate (Pilon-Smits, 2005). Several reports are showing that synthetic pyrethroid is biodegradable. Soil bacteria biodegraded cypermethrin to 3-phenoxybenzoic acid and a derivative of cyclopropanecarboxylic acid (Akbar et al., 2015). There was a report that *Plantago major*, a plant in the Plantaginaceae family, in combination with surfactant addition, 1% 2-hydroxypropyl-beta-cyclodextrin (HPßCD), could stimulate 70–80% removal of cypermethrin in soil (10 µg/g starting concentration) within 14 days (Aioub et al., 2019).

Phytoremediation is the most interesting method for managing pyrethroid-contaminated soil in agricultural areas of Thailand and using plants that grow well in Thailand. Napier grass (*Pennisetum purpureum*) is a popular forage crop for economic ruminants cultivated widely in central and northeastern parts of Thailand (Thongruang et al., 2021). This plant species was selected for use in the phytoremediation of pyrethroid-contaminated soil because this grass species can grow in low-quality soil and under a wide range of soil pH values, produces large amounts of biomass, and tolerates many pollutant-contaminants in soil (Osman et al., 2020; Ramadhan et al., 2015). Napier grass has been reported for phytoremediation of both organic and inorganic pollutants. For example, planting Napier grass in oil-contaminated soil for 40 days could decrease the total hydrocarbons in the soil (Bobor & Omosefe, 2019) and decrease the chlorobenzene in the soil within 150 days (Alvarenga et al., 2017). Co-planting between Napier grass and corn stimulated petroleum hydrocarbon removal to 83% within two weeks (Ayotamuno et al., 2006). The application of a synthetic surfactant has been reported to increase pyrethroid removal (Aioub et al., 2019). Normally, a surfactant increases the bioavailability of the pollutant and then increases microbial biodegradation and plant root uptake (Somtrakoon & Chouychai, 2021). Tween 20 has been reported to increase the absorption of organochlorine by *Miscanthus sinensis* (Mamirova et al., 2021). Even though there were no previous reports about synthetic pyrethroid phytoremediation with Napier grass, it could be possible to use this grass species for synthetic pyrethroid phytoremediation.

The phytotoxicity of pollutants normally retard plant growth and results in limited success of phytoremediation. Synthetic pyrethroid has also been reported to limit the growth of many plant species, such as corn and onion (Aveek et al., 2009). Plant growth regulators have often been reported to alleviate the toxicity of pollutants to plants. Among these, GA_3 is a plant growth regulator that could protect plants from pollutant toxicity and enhance the organic pollutant removal from soil. Ridge guard from seeds immersed in 1.0 mg/L GA_3 or watered with 10.0 mg/L GA_3 could increase the pyrene biodegradation after being planted in pyrene-contaminated soil for 30 days (Somtrakoon & Chouychai, 2022). Corn seed pretreatment with 0.1 mg/L GA_3 decreased hexachlorocyclohexane (HCH) contamination in soil (97.4% of initial concentration) when compared with soil planted with non-pretreated corn (35.7% of initial concentration) (Chouychai et al., 2015). Seeds immersion in 10.0 mg/L GA_3 could alleviate endosulfan toxicity to *Brassica chinensis* growing in 40 mg/kg alpha-endosulfan contaminated soil (Chouychai, 2012). In this study, the efficacy of Napier grass, cv. Pakchong 1 (*Pennisetum purpureum* x *Pennisetum* *americanum*) cuttings to enhance synthetic pyrethroid biodegradation and the effect of Tween 20, a synthetic surfactant, and GA_3 on synthetic pyrethroid phytotoxicity and biodegradation, were studied. It will be useful for improving phytoremediation efficacy for pesticide contamination in agricultural soil in Thailand.

MATERIALS AND METHODS

Plant and Soil Preparation

Napier grass cv. Pakchong 1 (*Pennisetum purpureum* x *Pennisetum americanum*) was obtained as commercial stem cuttings from Nong Bo Sub-district, Muang Ubon Ratchathani District, Ubon Ratchathani Province, Thailand. The stem cuttings were soaked in water for seven days after being received before being used. The soil used in this experiment was collected from Takhianluan Sub-district, Muang District, Nakhonsawan Province, Thailand. This soil was a sandy loam that was characterised previously (Chouychai et al., 2022). This soil was non-contaminated with 8 synthetic pyrethroids (bifenthrin, lambda-cyhalothrin, permethrin, cyfluthrin, cypermethrin, fenvalerate, and deltamethrin) confirmed by gas chromatography—micro-electron capture detector (GC-µECD) with a limit of detection as 0.01 mg/kg. This soil was spiked with the two commercial pyrethroid pesticides (Good Knock©, Thailand) that contained 10% (w/v) cypermethrin and 3% (w/v) deltamethrin (Delta©, Thailand) to a final concentration of cypermethrin, deltamethrin, permethrin, and fenvalerate in soil at 26.70, 116.20, 1.00 and 0.35 mg/kg, respectively. The details of the pyrethroid analysis will be explained below in the pyrethroid analysis.

Experimental Design

Gibberellic acid (GA_3, purity 90%, Sigma-Aldrich, China) was used in this study. There were two experimental designs, one for phytotoxicity and another for phytoremediation (Table 1). Each experimental design was completely randomise design (CRD) with one factor, six treatments per experiment and four replicates per treatment. A non-planted treatment with pyrethroid contamination and pyrethroid contamination + Tween 20 application was set as a control for pyrethroid biodegradation. After rooting, stem cuttings of Napier grass cv. Pakchong 1 in the GA_3 treatment were soaked in water or 0.01 mg/l GA_3 for 24 hours before being transferred to soil. This concentration of GA_3 has been reported to induce the growth of Napier grass cv. Pakchong 1 in non-contaminated soil (Phetsuwan et al., 2023). The pots used in this experiment were 7” in diameter, each containing 1 kg of dry soil. The cuttings were inoculated vertically into the soil. There were two stem cuttings per pot and four pots per treatment, and each pot was watered daily with 20 ml/pot. For the surfactant application treatments, Tween 20 (QRëC, New Zealand) with 1x of critical micelle concentration (CMC) were added to each pot at 20 ml/pot on the first day of the experiment. The surfactant used in this study was adapted from our previous work (Somtrakoon & Chouychai, 2021).

Table 1
Experimental design for pyrethroid phytotoxicity and pyrethroid phytoremediation

Treatment	Soil contamination	Planted with Napier grass	Gibberellic acid (GA_3) applied to plant cuttings	Surfactant application applied to soil
For pyrethroid phytotoxicity				
1	Non-contamination	Planted	None	No
2	Non-contamination	Planted	GA_3	No
3	Pyrethroid	Planted	None	No
4	Pyrethroid	Planted	GA_3	No
5	Pyrethroid	Planted	None	Tween 20
6	Pyrethroid	Planted	GA_3	Tween 20
For pyrethroid phytoremediation				
1	Pyrethroid	Non-planted	-	No
2	Pyrethroid	Non-planted	-	Tween 20
3	Pyrethroid	Planted	None	No
4	Pyrethroid	Planted	GA_3	No
5	Pyrethroid	Planted	None	Tween 20
6	Pyrethroid	Planted	GA_3	Tween 20

Plant Growth Analysis

Plants from each treatment were collected on day 20 after planting to determine the plant growth parameters, including the number of leaves and stems per plant, shoot length, root length, shoot fresh weight, shoot dry weight, root fresh weight, and root dry weight. The chlorophyll and carotenoid contents in the leaves were determined according to Lichtenthaler (1987). Briefly, 200 mg of fresh leaves were crushed with 100% acetone (Ajax Finechem, Australia), and the volume was adjusted to 15 ml. The absorbances were measured at 663.2, 646.8, and 470 nm, and the concentration of each pigment (μg/ml) was calculated as follows:

Chlorophyll *a* (Chl *a*) content = (12.5 *A663.2) - (2.79* A646.8)

Chlorophyll *b* (Chl *b*) content = (21.51*A646.8) - (5.1*A663.2)

Total chlorophyll content = Chl *a* + Chl *b*

Carotenoid content = (1000* A470) - (1.8* Chl *a*) - (85.02* Chl *b*)/198

In addition, the relative water content in the leaves was analysed by [(total fresh weight – bag weight) – dry weight)/(turgid weight – dry weight)]*100% as described in Sade et al. (2015). The leaf's proline content was analysed with spectrophotometry by measuring the absorbance of the leaf solution at 520 nm. The leaf solution was extracted by sulphosalicylic, following which it was reacted with the acid ninhydrin and extracted with toluene before being measured (John et al., 2008). The specific root length was calculated by root length/root dry weight (Calvelo Pereira et al., 2010), and the root-to-shoot ratio was calculated by root dry weight/shoot dry weight (Xu et al., 2018).

Pyrethroid Analysis

One kg of soil per pot was collected and sent for analysis of the pyrethroid pesticide concentration in the soil at the Central Laboratory Thailand, Ltd., Khon Kaen branch, using an in-house method based on the QuEChERS method using GC-μECD with a limit of detection as 0.01 mg/kg and in addition, dried shoots and roots of Napier grass cv. Pakchong 1 were collected and sent for analysis of the pyrethroid pesticide accumulation in plant tissue at the Central Laboratory Thailand, Ltd., Khon Kaen branch using an in-house method based on the QuEChERS method using GC-μECD with a limit of detection of 0.01 mg/kg. Each synthetic pyrethroid's bioconcentration factors (BCF) were calculated for each synthetic pyrethroid concentration in the harvested plant/each synthetic pyrethroid concentration in planted soil. A BCF higher than one showed the potential of plants to phytoaccumulate (Hammami et al., 2016).

Statistical Analysis

One-way analysis of variance (ANOVA) and Duncan's tests were used for variance analysis and pairwise comparison.

RESULTS AND DISCUSSION

Effect of Gibberellic Acid and Tween 20 on Napier Grass cv. Pakchong 1 Growth in Pyrethroid Contaminated Soil

Synthetic pyrethroids were significantly toxic to the growth of Napier grass cv. Pakchong 1. Shoot length, shoot fresh weight, and shoot dry weight of Napier grass grown in pyrethroid-contaminated soil were significantly lower than those grown in non-contaminated soil (Table 2). The shoot length of Napier grass cv. Pakchong 1 grown in non-contaminated soil was 44.5 ± 0.61 cm, while the shoot length of those grown in pyrethroid-contaminated soil was 31.0 ± 0.91 cm. In addition, the root length, root fresh weight, root dry weight, and root-to-shoot ratio of Napier grass growing in pyrethroid-contaminated soil were decreased when compared with those growing in non-contaminated soil. The root thickness tended to decrease, as shown by increasing the specific root length from 3.18 ± 0.06 m/g in non-contaminated soil to 4.82 ± 0.35 m/g (Table 3 and Figure 1).

Soaking with GA_3 could significantly increase the shoot length, shoot fresh weight, and shoot dry weight of Napier grass grown in pyrethroid-contaminated soil compared with plants not stimulated with GA_3. This stimulating effect on shoot growth was not seen when comparing cuttings that were soaked and not soaked with GA_3 in non-contaminated soil. The shoot length of Napier cuttings soaked and not soaked with GA_3 in pyrethroid-contaminated soil were 44.4 ± 0.47 and 31.0 ± 0.91 cm, respectively, while those in non-contaminated soil were 38.0 ± 0.41 and 44.5 ± 0.61 cm, respectively (Table 1). However, the positive effect of GA_3 on root growth was less than on shoot growth. Soaking with GA_3 significantly increases only the root dry weight of Napier grass grown in pyrethroid-contaminated soil. This effect of GA_3 is similar to our previous study with hexachlorocyclohexane (HCH) (Chouychai et al., 2015) and polycyclic aromatic hydrocarbons (PAHs) (Somtrakoon & Chouychai, 2022). GA_3 is a plant hormone that controls plants' normal shoot and root growth (Hedden & Sponsel, 2015), and the application of exogenous GA_3 in this study was adequate to induce shoot growth of Napier grass in pyrethroid-contaminated soil.

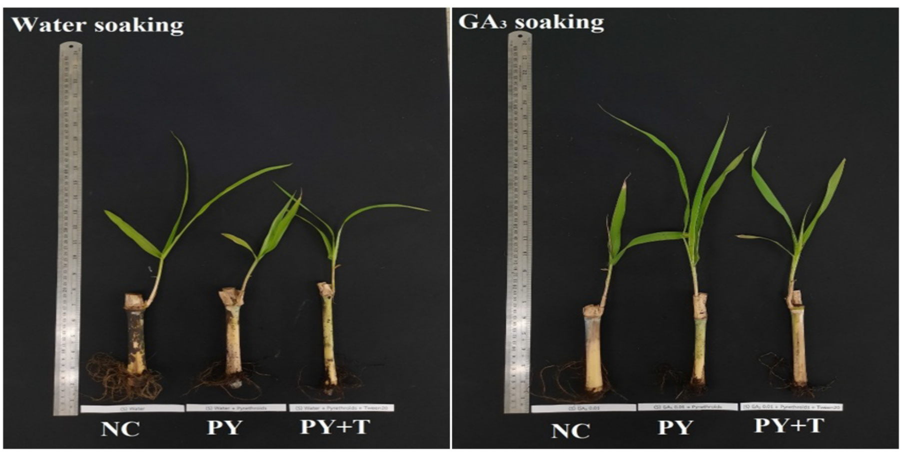

Figure 1. Shoot and root of Napier grass cv. Pakchong 1 soaking in water or 0.01 mg/L gibberellic acid (GA_3) and growing in different soil that was non-contaminated soil (NC), pyrethroid-contaminated soil with (PY + T) or without Tween 20 addition (PY) for 20 days

The positive effect of Tween 20 on Napier grass was less than GA_3. In addition, Tween 20 only increased the shoot length and shoot dry weight and did not enhance the root growth of Napier grass soaked with water and growing in pyrethroid-contaminated soil. It contrasts with Triton X-100 and Tween 80 in addition to *Impatiens balsamina* planted in 100 mg/kg anthracene and fluoranthene-contaminated soil, in which the shoot growth of the plants exposed to both surfactants was decreased (Somtrakoon & Chouychai, 2021). Tween 20, in addition to pyrethroid-contaminated soil and planted with GA_3-soaked cuttings, did not enhance the growth of Napier grass over that which received only GA_3. Depending on shoot and root growth, GA_3 soaking was appropriate to improve the Napier grass health in pyrethroid-contaminated soil.

Growth in pyrethroid did not affect the chlorophyll *b*, carotenoid, and relative water contents of Napier grass, and treatment with GA_3 or Tween 20 did not enhance these plant traits. For example, the carotenoid contents

Table 2
Shoot growth of Napier grass receiving gibberellic acid (GA_3) and Tween 20 and growing in non-contaminated soil or pyrethroid-contaminated soil for 20 days

Treatment	Shoot length (cm)	Shoot fresh weight (g)	Shoot dry weight (g)	Number of leave/cutting	Number of shoot/cutting
NC + Water-Np	44.5 ± 0.61 a	25.5 ± 0.52 a	14.7 ± 0.67 a	3.2 ± 0.25 a	1.0 ± 0.00
NC + GA_3-Np	38.0 ± 0.41 bc	26.7 ± 0.60 ab	15.2 ± 1.38 a	3.5 ± 0.29 a	1.0 ± 0.00
Pyrethroid + Water-Np	31.0 ± 0.91 d	19.3 ± 0.38 e	10.4 ± 0.36 b	2.8 ± 0.25 a	1.0 ± 0.00
Pyrethroid + Tween 20 + Water-Np	37.0 ± 0.41 c	21.4 ± 0.95 de	14.5 ± 0.56 a	3.0 ± 0.00 a	1.0 ± 0.00
Pyrethroid + GA_3-Np	44.4 ± 0.47 a	23.9 ± 0.90 bc	13.4 ± 0.66 a	3.2 ± 0.25 a	1.0 ± 0.00
Pyrethroid + Tween 20 + GA_3-Np	39.0 ± 0.71 b	22.5 ± 0.75 cd	14.3 ± 0.42 a	3.2 ± 0.25 a	1.0 ± 0.00

Note. Different lowercase letters showed significant difference ($P < 0.05$) between different treatment in same column; NC = Non-contaminated soil; Water-NP = Water soaking Napier grass cutting, GA_3-Np = GA_3 soaking Napier grass cutting

Table 3
Root growth of Napier grass receiving gibberellic acid (GA_3) and Tween 20 and growing in non-contaminated soil or pyrethroid-contaminated soil for 20 days

Treatment	Root length (cm)	Root fresh weight (g)	Root dry weight (g)	Root-to-shoot ratio	Specific root length (m/g)
NC + Water-Np	25.2 ± 0.63 a	0.76 ± 0.03 a	0.08 ± 0.003 a	0.005 ± 0.000 a	3.18 ± 0.06 b
NC + GA_3-Np	23.0 ± 0.41 b	0.68 ± 0.04 b	0.08 ± 0.003 a	0.005 ± 0.000 a	2.99 ± 0.16 b
Pyrethroid + Water-Np	16.1 ± 0.31 c	0.34 ± 0.03 c	0.03 ± 0.003 d	0.003 ± 0.000 c	4.82 ± 0.35 a
pyrethroid + Tween 20 + Water-Np	16.2 ± 0.25 c	0.33 ± 0.02 c	0.04 ± 0.003 cd	0.003 ± 0.000 c	4.23 ± 0.34 a
Pyrethroid + GA_3-Np	16.2 ± 0.25 c	0.39 ± 0.02 c	0.05 ± 0.004 bc	0.004 ± 0.000 b	3.27 ± 0.33 b
pyrethroid + Tween 20 + GA_3-Np	17.0 ± 0.41 c	0.40 ± 0.02 c	0.06 ± 0.006 b	0.004 ± 0.000 b	3.15 ± 0.39 b

Note. Different lowercase letters showed significant difference ($P < 0.05$) between different treatment in same column; NC = Non-contaminated soil; Water-NP = Water soaking Napier grass cutting, GA_3-Np = GA_3 soaking Napier grass cutting

in the leaves of Napier grass were between 0.12–0.15 mg/g FW for plants grown in non-contaminated soil and between 0.07–0.16 mg/g FW for plants growing in pyrethroid-contaminated soil (Table 4). However, pyrethroid contamination decreased the chlorophyll *a* and total chlorophyll contents and increased the proline content in the leaves of Napier grass. Both GA_3 and Tween 20 applications increased the chlorophyll *a* and total chlorophyll contents in the leaves of Napier grass grown in pyrethroid-contaminated soil. However, only GA_3-treated Napier grass grown in pyrethroid-contaminated soil contained proline in the leaves (11.5 mg/g FW) at the same level as plants grown in non-contaminated soil (9.8 mg/g FW). Application of Tween 20 could decrease the proline content (14.9 mg/g FW) when compared with plants grown in pyrethroid-contaminated soil that did not receive Tween 20 (19.4 mg/g FW), but the proline content was still significantly higher than that growing in non-contaminated soil (9.8 mg/g FW) (Table 4).

Table 4
Chlorophyll, carotenoid, relative water, and proline content in leaves of Napier grass receiving gibberellic acid (GA_3) and Tween 20 and growing in non-contaminated soil or pyrethroid-contaminated soil for 20 days

Treatment	Chlorophyll *a* content (mg/g FW)	Chlorophyll *b* content (mg/g FW)	Total chlorophyll content (mg/g FW)	Carotenoid content (mg/g FW)	Relative water content (%)	Proline content (µg/g FW)
NC + Water-Np	0.67 ± 0.02 a	0.27 ± 0.03 a	0.94 ± 0.04 a	0.15 ± 0.02 a	55.4 ± 3.84 a	9.8 ± 0.74 c
NC + GA_3-Np	0.60 ± 0.07 a	0.35 ± 0.07 a	0.95 ± 0.03 a	0.12 ± 0.04 a	59.9 ± 4.18 a	10.1 ± 0.37 c
Pyrethroid + Water-Np	0.38 ± 0.05 b	0.17 ± 0.03 a	0.54 ± 0.03 b	0.07 ± 0.01 a	54.7 ± 10.44 a	19.4 ± 0.81 a
Pyrethroid +Tween 20 + Water-Np	0.60 ± 0.07 a	0.19 ± 0.02 a	0.79 ± 0.09 a	0.15 ± 0.02 a	67.9 ± 9.50 a	14.9 ± 0.98 b
Pyrethroid+ GA_3-Np	0.62 ± 0.06 a	0.24 ± 0.05 a	0.86 ± 0.07 a	0.14 ± 0.01 a	72.4 ± 12.48 a	11.5 ± 1.95 c
Pyrethroid + Tween 20 + GA_3-Np	0.68 ± 0.06 a	0.28 ± 0.08 a	0.96 ± 0.06 a	0.16 ± 0.03 a	61.8 ± 7.12 a	12.5 ± 0.68 bc

Note. Different lowercase letters showed significant difference ($P < 0.05$) between different treatment in same column; NC = Non-contaminated soil; Water-NP = Water soaking Napier grass cutting, GA_3-Np = GA_3 soaking Napier grass cutting

The toxicity of synthetic pyrethroid to Napier grass was the same as that reported for *Allium cepa*, *Lathyrus sativas*, and *Zea mays* exposed to 0.2-0.8 g/L cypermethrin 12 hr before germination. Cypermethrin significantly decreased the shoot and root length, mitotic index, chlorophyll content, and moisture content for all three plants (Aveek et al., 2009). GA_3 soaking could alleviate these toxicities to Napier grass, including decreasing the proline content in leaves. It indicates that GA_3 could decrease the pyrethroid stress to plants, which is advantageous for phytoremediation. The proline content in plants often increases when exposed to stress, and it has been reported to function as a protein stabiliser, hydroxyl radical, singlet oxygen scavenger and inhibitor of lipid peroxidation (Siddiqui et al., 2011). Reports show that GA_3 could alleviate pollutant phytotoxicity and increase biodegradation of organic pollutants. GA_3 stimulated root growth, and then the healthy root could stimulate the growth and function of soil bacteria that could degrade the pollutant in the rhizosphere via the rhizodegradation process (Alagić et al., 2015) or plant-microbe interactions in the root zone (Chaudhry et al., 2005). Applying GA_3 alone or in combination with Tween 80 could increase the plant dry weight of *Tagetes patula* grown in benzo[a]pyrene and cadmium-contaminated soil (Sun et al., 2013).

Effect of Gibberellic Acid and Tween 20 on Pyrethroid Phytoremediation with Napier Grass cv. Pakchong 1

There were four pyrethroid pesticide groups found in contaminated soil, which were 26.7 mg/kg cypermethrin, 116.2 mg/kg deltamethrin, 1.00 mg/kg permethrin, and 0.35 mg/kg fenvalerate, at the beginning of the experiment (Table 5). After 20 days, the concentrations of cypermethrin, deltamethrin, and felvalorate decreased significantly in non-planted soil, while the concentration of permethrin in the soil did not decrease significantly. In addition, the accumulation of cypermethrin, deltamethrin, and permethrin in the shoots and roots of Napier grass cv. Pakchong 1 is limited as the bioconcentration factor was below 1, while fenvalerate was not found in any plant tissue (Table 6). Pyrethroid accumulation was not found in Napier grass growing in non-contaminated soil. These results contrast with aquatic plants that could remove synthetic pyrethroid from water by 58–76% within seven days (Riaz et al., 2017). It showed that the decrease in the synthetic pyrethroid in soil within 20 days did not occur by plant accumulation but depended on the activity of indigenous soil bacteria.

There has been a report that cypermethrin was degraded more rapidly in non-sterilised soil (with soil bacteria) than in sterilised soil (low soil bacteria by autoclaving at 120ºC for 20 min on two consecutive days). After 28 days, 2.89% of cypermethrin (starting concentration = 10 mg/kg) was removed in sterilised soil, while 8.41% of cypermethrin was removed in non-sterilised soil (Xie & Zhou, 2008). These bacteria could degrade cypermethrin, deltamethrin, and fenvalorate but could not degrade permethrin. Adding Tween 20 into non-planted soil or that planted with Napier grass did not enhance any pyrethroid removal in the soil. This effect was the same as with Tween 20 addition into phenanthrene-contaminated soil in which Tween 20 inhibited phenanthrene biodegradation (Zhou et al., 2008). The failure of the surfactant application has been reported. Tween 80 addition did not enhance PAH-phytoremediation by *Impatiens balsamina* but could enhance PAH biodegradation in non-planted soil when used with salicylic acid (Somtrakoon & Chouychai, 2021).

GA_3-stimulated Napier grass cutting did not exhibit any enhanced pyrethroid removal.

When comparing with cypermethrin biodegradation in soil planted with *Plantago major* (60% removal) for 14 days and a starting concentration of 10 mg/kg (Aioub et al., 2019), Napier grass cv. Pakchong 1 soaked in GA_3 was more effective with 66.3% cypermethrin removal (starting concentration was 26.7 mg/kg and planted for 20 days), while the effect of Napier grass cv. Pakchong 1 soaked in water was the same as *Plantago major* (59.6% removal). However, planting Napier grass cv. Pakchong 1 soaked with GA_3 or Tween 20 was less effective than *Plantago major* and liquid silicon dioxide addition (80% removal) (Aioub et al., 2019). The cultivation of Napier grass was stimulated with GA_3 and could tolerate synthetic pyrethroid-contaminated soil well, but the capacity to remove pyrethroid from the soil is limited and should be improved.

Table 5
Pyrethroid remaining in soil after planting with Napier grass for 20 days

Treatment	Cypermethrin (mg/kg)	Deltamethrin (mg/kg)	Permethrin (mg/kg)	Fenvalerate (mg/kg)
Starting concentration	26.7 ± 4.89 a	116.2 ± 26.4 a	1.00 ± 0.41 a	0.35 ± 0.06 a
No plant	14.0 ± 3.56 b	53.0 ± 20.3 b	0.7 ± 0.18 a	0.18 ± 0.04 b
No plant + Tween 20	17.8 ± 0.37 ab	97.9 ± 1.42 ab	1.0 ± 1.00 a	0.23 ± 0.02 b
Napier-water	10.8 ± 0.94 b	66.5 ± 7.20 b	0.8 ± 0.07 a	0.19 ± 0.02 b
Napier-water + Tween 20	12.0 ± 0.89 b	70.7 ± 5.02 b	0.9 ± 0.06 a	0.19 ± 0.01 b
Napier-GA_3	9.0 ± 0.49 b	56.2 ± 1.23 b	0.6 ± 0.02 a	0.16 ± 0.00 b
Napier-GA_3 + Tween 20	13.5 ± 2.37 b	91.1 ± 15.52 ab	0.9 ± 0.13 a	0.20 ± 0.04 b

Note. Different lowercase letters showed significant difference ($P < 0.05$) between different treatment in same column; GA_3 = Gibberellic acid

Table 6
Pyrethroid accumulation in plant tissue of Napier grass after planting for 20 days

Treatment	Cypermethrin	Deltamethrin	Permethrin
Shoot concentration (mg/kg)			
Napier-water	0.11	0.14	0.04
Napier-water + Tween 20	0.04	B.D.	0.04
Napier-GA_3	0.23	0.40	0.07
Napier-GA_3 + Tween 20	0.05	0.07	B.D.
Root concentration (mg/kg)			
Napier-water	0.11	0.20	0.05
Napier-water + Tween 20	0.03	0.05	0.01
Napier-GA_3	B.D.	0.01	B.D.

Table 6 *(Continue)*

Treatment	Cypermethrin	Deltamethrin	Permethrin
Napier-GA_3 + Tween 20	0.01	0.01	B.D.
Bioconcentration factor			
Napier-water	0.020	0.005	0.106
Napier-water + Tween 20	0.006	0.001	0.056
Napier-GA_3	0.025	0.007	0.111
Napier-GA_3 + Tween 20	0.004	0.001	-

Note. GA_3 = Gibberellic acid

CONCLUSION

Napier grass cv. Pakchong 1 could grow in synthetic pyrethroid-contaminated soil, but the shoot, root growth, and pigment content in leaves decreased. Soaking stem cuttings of Napier grass in 0.01 mg/L GA_3 before planting in synthetic pyrethroid-contaminated soil could stimulate growth and alleviate plant stress. Napier grass cv. Pakchong 1 enhanced the removal of cypermethrin, deltamethrin, and fenvalerate significantly within 20 days, and it did not accumulate all pyrethroid pesticides within plant tissue. In addition, Tween 20 addition to soil and GA_3-soaked cuttings did not stimulate synthetic pyrethroid removal from soil. The application of GA_3 is appropriate to induce the growth of Napier grass, but the method to enhance pyrethroid in Napier grass planting soil should be further developed.

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