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Eating Fresh Strawberry Fruits Free from Pesticide Residues by Using 28-Homobrassinolide.

Raed S Shehata

Department of Agriculture, Ministry of Agriculture and land reclamation, Damanhour, Egypt. https://orcid.org/0000-0002-7086-3982
Correspondence: raedsalem8882001@gmail.com

Abstract

One of the most important goals is producing healthy food to eliminate the harmful effects of pesticide residues in plant foods such as grains, vegetables and fruits. There is no doubt that applying good farming practices such as relying on biological pest control and plant growth regulators to achieve the goal of eliminating the dangers of pesticides residues to human health. This is proven by our study using GC-MSMS and LC-MSMS through a full scan of samples of fresh Strawberry fruits (Fragaria × anannasa Duch) var 'Sensation' from the private agricultural field were obtained from badr district, el Beheira Governorate, Egypt. All of which were proven by analysis to be free of the pesticide residues. The results showed that 28-Homobrassinolide at 0.05 mg/l in a greatest decrease in concentrations of pesticide residues (imidacloprid, fenpyroximate, difenoconazole, azoxystrobin, fludioxonil and Lambda-cyhalothrin). Overall, 28-Homobrassinolide at 0.05 mg/l improved detoxification of pesticide residues from fresh strawberry fruits.

Key Words: *Pesticide residues, Strawberries, GC-MSMS, LC-MSMS, 28-Homobrassinolide.*

Introduction

Strawberry (Fragaria × ananassa) known as false fruit is grown in temperate, tropical and subtropical regions of the world and is a member of the Rosaceae family (Trejo-Téllez & Gómez-Merino, 2014). Its excellent flavour, taste and nutritional value made (Rajwana et al., 2017). Strawberry (Fragaria × ananassa Duch.) Is a vital and economical fruit crop that can be grown in a wide range of environments. Customers attracted to its appealing to flavour and aroma, its broad acceptance as a preferred fruit (Lewers et al., 2020; Fecka et al., 2021).

Pesticides known as Toxic chemicals are frequently employed to manage diseases, weeds and pests in order to guarantee crop quality and productivity (Collotta et al., 2013). Because of their negative impact on ecosystems, pesticide residues are now more widely acknowledged as pollutants in the environment (Song et al., 2007). Brassinosteroids (BRs) diversified class of steroid phytohormones (polyhydroxysteroids), (BRs) have a wide range of biological roles (Nolan et al., 2020; Qiao et al., 2022). BRs play important roles in development, plant growth, adaptation to environmental stress and fruit quality (Siddiqi & Husen, 2021; Zhang & Yang, 2021; shehata, 2024). In various types of crops, such as cucumber, mustard, tomato (Lycopersicon esculentum), strawberry (Fragaria ananassa), grapes, and tea (Camellia sinensis), exogenous application of the BR analogue 24-epibrassinolide (EBR) decreased pesticide residues (Sharma et al., 2019; Wang et al., 2017; Xia et al., 2006, 2009; Zhou., 2015). The objectives of this study were to use 28-Homobrassinolide as an exogenous spray to enhance detoxification of pesticide residues from fresh strawberry fruits. For growing healthy food free from any pesticide residues to protect humanity from potential illnesses.

Materials and Methods

Chemicals and Reagents

Certified reference material of GC-MSMS Pesticide combinations including 9 multi pesticide residue mixture as listed below.

GC Multiresidue Pesticide Standard - 185 components including,

Allidochlor, Dichlofluanid, Biphenyl, Mevinphos, 3,4-Dichloroaniline, Etridiazole, Pebulate, N-(2,4-Dimethylphenyl)Formamide ,Captafol, Methacrifos, Chloroneb, 2-Phenylphenol, Pentachlorobenzene, Tecnazene, Propachlor, Diphenylamine, 2,3,5,6-Tetrachloroaniline, Cycloate, Chlorpropham, Ethalfluralin, Trifulralin, Benfluralin, Sulfotep, Diallate , Phorate, Alpha -HCH, Diallate isomer, Hexachlorobenzene, Dichloran, Pentachloroanisole, Atrazine, Clomazone, Beta-HCH, Gamma-HCH(lindane), Terbuthylazine, Terbufos, Profluralin, Quintozene, Propyzamide, Disulfoton, Tefluthrin, Delta-HCH, Isazofos, Tri-allate, Chlorothalonil, Endosulfan-alpha, Prapanil, Dimethachlor, Acetochlor isomer, Vinclozolin, Chlorpyrifos-Methyl, Cypermethrin, Tolclofos-Methyl, Alachlor, Acetochlor, Heptachlor, Metalaxyl, Fonofos, Pentachlorobenzonitrile, Pyrimethanil, Diazinon, Etofenprox, Fluchloralin, Fenchlorphos, Prodiamina, Fenitrothion, Pirimiphos-methyl, Malathion, Methyl-Pentachlorophenyl sulfide, Dichlobenil, Metolachlor, Anthraquinone, Fenthion, Aldrin, Chlorpyrifos, Parathion, Triadimefon, 4,4-Dichlorobenzophenone, Chlorthal-dimethyl, Fenson, Diphenamid, Pirimiphos-ethyl, Bromophos-Methyl, Isopropalin, Cyprodinil, Isodrin, Metezachlor, Pendimethalin, Penconazole, Chlozolinate, Bioallethrin, Fipronil, Heptachlor-exo-epoxide, Tolyfluanid, Chlorbenzilate, Bromfenvinphos-Ethyl, Quinalphosl, Triadimenol, Folpet, Procymidone, Chlorbenside, Bromophos-Ethyl, Cis-Chlordane, 2,4-DDE, Paclobutrazol, Cis-Tetrachlorvinphos, Fenamiphos, Endosulfan-beta, Flutriafol, Trans-Chloridane, Chlorfenvinphose, Flutolanil, Cis-Nonachlor , Iodofenphos, Prothiophos, Fludioxonil, Profenfos, Pretilachlor, Oxadiazon, 4,4-DDE, Oxyfluorfen, Myclobutanil, Dieldrin, Flusilazole, Bupirimate, 2,4-DDD, Fluazifop-P-butyl, Chlorfenapyr, Nitrofen, Chlorobezilate, Endrin, Endosulfan Ether, 4,4-DDD(TDE), Ethion, 2,4-DDT, Chlorthiophos, Trans-Nonachlor, Triazophose, Sulprofos, Enderin Aldehyde, Enderin ketone, Carfentrazone-Ethyl, 4,4-methoxychlor-olefin, Carbophenothion, Norflurazon, Edifenphose, Lenacil, 4,4-DDT, Endosulfan total, Hexazinone, o,p-Methoxychlor-olefin, Methoxychlor(DMTD), Propargite, Piperonyl butoxide, Resmethrin, Pyridaphenthion, Cis-Bifenthrin, Tetramethrin, Bromopropylate, Phosmet, EPN, Fenpropathrin, Tebufenpyrad, Phenothin, Phenothrin, Tetradifon, Pyriproxyfen, Azinophos-Methyl, Leptophos, Iambda-Cyhalothrin, Mirex, Pyrazophos, Fenarimol, Azinophose-Ethyl, Pyraclofos, Cis-Permethrine, Trans-Permethrin, Pyridaben, Coumaphos, Fluquinconazole, Cyfluthrin, Fenvalerate, Flucythrinate, isomerFlucythrinate and Fluridone.

LC Multiresidue Pesticide Standard - 45 components including:

Rotenone, Spirodiclofen, azoxystrobin, Flufenoxuron, Indoxacarb, Chlorfluazuron, Linuron, Carbofuran, Bendiocarb, Mevinphors, Ametryn, Dimethoate, Carboxin, 3-Hydroxycarbofuran, Dicrotophos, Primicarb, Forchlorfenuron, Clothianidin, Thiacloprid, imidacloprid, Dithofencarb, Chloroxuron, Thiamethoxam, Spiroxamine, Propham, Tricyclazole, Cycluron, Carbaryl, Thiabendazole, Promecarb, Propoxur, difenoconazole, Fenoxycarb, Diflubenzuron, Cyazofamid, Benalaxyl, Boscalid, Triflumeron, Propargite, Spiromesifen, Spirotetramat, fenpyroximate, Dimethomoroph, Spinosad, Spinetoram, and Emamectin benzoate were purchased from CAP- CHEM (Augsburg, Germany), with certified purity ranging from 97% to 99.9%. Acetonitrile, ammonia solution, sodium chloride, acetic acid, triphenylphosphate (TPP), and ethoprophos were obtained from chem- lab (Belgium), and QuEChERS extraction mix kit from CHROMABOND (Valencienner St. 11, 52355 Duren, Germany). All the organic solvents used were higher performance liquid chromatography (HPLC) grade.

Field experiment

The present study was carried out during in two successive 2023 and 2024 on strawberry (Fragaria X ananassa Duch) var "Sensation", grown under a drip irrigation system in a private field at badr district, el Beheira Governorate, Egypt. Soil texture was sandy and drip irrigation system was adopted. Strawberry plants had been under the standard agriculture practices throughout the seasons. Three replications were used for each treatment. The treatments included: control (tap water spray), 28-Homobrassinolide at (0.025 mg/l) and 28-Homobrassinolide at (0.05 mg/l). The non-ionic surfactant Top film at 0.05% (V/V) was added to all treatments to reduce the surface tension and to increase the contact angle of sprayed droplets. These treatments were arranged in Analysis of Variance (ANOVA) for Randomized Complete Block Design (RCBD).

Samples collection

Fresh strawberry fruits were picked at mid-December after 5 days of pre-harvest treatments, when bright red color (3/4 of surface showing red) at the same ripening stage. The fruits were selected based on the uniform color, same size and no pests, as well as the absence of blemishes or disease, with no physical damage. The samples included 1kg from fresh strawberry fruits for each replicate for pesticide residue analysis. The samples were collected from private field at badr district, el Beheira Governorate, Egypt. The sampling was performed in accordance with the general principles and methods of the European Commission (EC) directive 2002/63/EC for establishing MRLs in food commodities (EC, 2002). All the samples were placed in sterile polythene bags, in an ice box, to avoid contamination and deterioration, labeled, and transported to the laboratory for processing. The homogenized samples were analyzed immediately.

Sample Extraction and Clean-Up

The extraction and clean-up method used was based on QuEChERS (quick easy cheap effective rugged and safe) sample preparation method for pesticides (BS EN 15662:2018). An aliquot of 10 g of homogenized sample was placed in a 50 mL centrifuge, and 10 mL of acetonitrile was added. The mixture was vortexed for one minute, followed by adding 4 g of magnesium sulphate anhydrous, 1g trisodium citrate di hydrate, 0.5 g disodium hydrogen citrate and 1 g of sodium chloride. The clean-up was carried out by transferring 3 ml of supernatant into another tube containing 75 mg of primary and secondary amine (PSA), 75 mg of C18 Sorbent (for wheat) and 450 mg of magnesium sulphate. After agitation and centrifugation, the aliquots of the extract were reconstituted to 1.5 mL vial for gas chromatography (GC-MS/MS) (Mustapha et al., 2017).

Solutions and Standards

From Reference Standard pesticide mixtures (9 Mixture 100ppm each compound for GC-MS/MS and 10 mixtures 100ppm each compound for LC-MSMS), stock solutions (1 ppm) were prepared in acetonitrile for GC-MSMS and LC-MSMS analysis. Multi-residue working solutions containing pesticides analyzed by GC-MS/MS and LC-MSMS were prepared in acetonitrile at a concentration of 5 – 100 ppb for each pesticide to create calibration curves.

GC-MSMS Analysis

GC-MS/MS analysis was proceeding using a gas chromatography (GC-MSMS- TSQ9610 Mass Spectrometer, Thermofisher, USA), With Trace gold GC column TG-5MS, length 30 m, internal diameter 0.25mm, film 0.25µm and temperature -60 to 330/350°. Sample injection was performed in the splitless mode, with an injector temperature of 250 °C and an interface temperature of 280 °C. The temperature of the oven was programed from an initial value of 80 °C for 1 min, ramped to 160 °C at 10 °C min^{-1} for 2 min, and to 280 °C at 12 °C min^{-1} for 6 min. Helium was used as a carrier gas with a constant flow rate of 1.2 mL min^{-1}. Electron ionization was used at – 70 eV in selective reaction monitoring (SRM) and full-scan modes between 50 m/z and 550 m/z for the detection of different analyses.

LC-MSMS Analysis

LC-MS/MS analysis was proceeding using a liquid chromatography (LC- MSMS-SCIEX QTRAP 4500, Canada), with Synergi 2.5µm fusion – RP100A, LC column 50*2 mm. C-18 analytical column. The sheath gas temperature was kept at 400 °C. Deionized water containing 0.1% formic acid and ammonium formate 5 mM (mobile phase A), and methanol and (5mM) ammonium formate dissolved in deionized water (95:5, v/v) containing 0.1% formic acid (mobile phase B) were used for the gradient program, which started with 100% A for 1 min and was linearly decrease to 0 % A and 100% B over 13 min. then decrease B to 0 % and 100% A over 6 min. The column temperature was kept at 40 °C, and the injection volume was 3µL with a constant flow rate of 0.4 mL_min^{-1}. For each compound, two multi reaction monitoring (MRM) transitions were monitored. The pesticides Propham, Tricyclazole, Cycluron, Carbaryl, Thiabendazole, Promecarb, Propoxur, Linuron, Carbofuran, Bendiocarb, Mevinphors, Ametryn, Dimethoate, Carboxin, 3-Hydroxycarbofuran, Dicrotophos, Primicarb, Forchlorfenuron, Clothianidin, Thiacloprid, Dithofencarb, Chloroxuron, Thiamethoxam, Spiroxamine, Fenoxycarb, Diflubenzuron, Cyazofamid, Benalaxyl, Boscalid, Triflumeron, Propargite, Spiromesifen, Spirotetramat, Dimethomoroph, Rotenone, Spirodiclofen, Flufenoxuron, Indoxacarb, Chlorfluazuron, Spinosad, Spinetoram, and Emamectin benzoate were analyzed with LC-MS/MS. The results of all investigated sample for above 42 pesticides were less than LOQ (5 ppb). The results indicate the safety of organic vegetables and fruits for human health because of no pesticide residue (Willer and Lernoud, 2017; Apaolaza et al., 2018).

Experimental

The analysis precured as shown in the standard method of Britch standard institute (BS EN 15662:2018). Multimethod for the determination of pesticide residues using GC- and LC-based analysis following acetonitrile (Thermofisher) extraction and clean-up by dispersive solid phase extraction (SPE). Modular QuEChERS-method.

Chromatography techniques

GC-MSMS Thermofisher TSQ9610 With Trace gold GC column TG-5MS, length 30 m, internal diameter 0.25mm, film 0.25µm and temperature -60 to 330/350° c. PrO-Analytical Centrifuges.

Data analysis

Statistical comparisons of differences were made by analysis of variance (ANOVA). To compare the means between different treatments concentrations and Pesticide Residues, the Least Significant Difference (LSD) test was conducted at the 0.05 level of probability, the differences between treatments concentrations and Pesticide Residues were designated by different letters. According to (Snedecor & Cochran, 1980). The data was analyzed using (SAS, 2009).

Results

Changes in pesticide residues (Imidacloprid, Fenpyroximate and Lambda-cyhalothrin) of "Sensation" Strawberry fruits in response to used applications of 28-Homobrassinolide were reported in (Table 1, Figure 1, Figure 2 and Figure 3). The data confirmed the previous trend that pesticide residues (Imidacloprid, Fenpyroximate and Lambda-cyhalothrin) were significantly decreased by two applications compared with control. Moreover, the greatest decrease in pesticide residues (Imidacloprid, Fenpyroximate and Lambda-cyhalothrin) were obtained with 28-Homobrassinolide at 0.05 mg/l in both seasons. The use of 28-Homobrassinolide with the lower concentration at 0.025 mg/l was able to cause a significant decrease in pesticide residues (Imidacloprid, Fenpyroximate and Lambda-cyhalothrin) as compared with control.

The influence of pre-harvest treatments of 28-Homobrassinolide on pesticide residues (Difenoconazole, Azoxystrobin and Fludioxonil) of "Sensation" Strawberry fruits whether high or low concentrations were reported in (table 2, Figure 4, Figure 5 and Figure 6). The data showed that pesticide residues Difenoconazole, Azoxystrobin and fludioxonil were disappeared from strawberry fruits samples when applied 28-Homobrassinolide (at 0.05 mg/l) in both seasons. When 28-Homobrassinolide was applied (at 0.025 mg/l), it gave low pesticide residues (Difenoconazole, Azoxystrobin and Fludioxonil) as compared with control.

Table 1. The effect of pre-harvest applications of various 28-Homobrassinolide concentration on Pesticide Residues (Imidacloprid, Fenpyroximate and Lambda-cyhalothrin) of "Sensation" Strawberry fruits during the two seasons 2023 and 2024.

Treatments	Pesticide Residues (active ingredient of pesticides)					
	Imidacloprid (mg/kg)		Fenpyroximate (mg/kg)		Lambda-cyhalothrin (mg/kg)	
	2023	2024	2023	2024	2023	2024
Control	0.023[a]	0.030[a]	0.111[a]	0.121[a]	0.041[a]	0.052[a]
28-Homobrassinolide at 0.025 mg/l	0.010[b]	0.020[a]	0.108[a]	0.100[a]	0.035[a]	0.048[b]
28-Homobrassinolide at 0.05 mg/l	0.005[b]	0.005[b]	0.051[b]	0.048[b]	0.006[b]	0.009[c]
LSD at 0.05	0.008	0.011	0.011	0.051	0.010	0.002

Values in each column when accompanied with similar letters, were not significantly different by using the least Significant Difference at 0.05 for comparing the means.

Table 2. The effect of pre-harvest applications of various 28-Homobrassinolide concentration on Pesticide Residues (Difenoconazole, Azoxystrobin and Fludioxonil) of "Sensation" Strawberry fruits during the two seasons 2023 and 2024.

Treatments	Pesticide Residues (active ingredient of pesticides)					
	Difenoconazole (mg/kg)		Azoxystrobin (mg/kg)		Fludioxonil (mg/kg)	
	2023	2024	2023	2024	2023	2024
Control	0.269[a]	0.254[a]	0.535[a]	0.622[a]	0.012[a]	0.014[a]
28-Homobrassinolide at 0.025 mg/l	0.043[b]	0.114[b]	0.064[b]	0.096[b]	0.005[b]	0.005[b]
28-Homobrassinolide at 0.05 mg/l	0.000[c]	0.000[c]	0.000[c]	0.000[c]	0.000[c]	0.000[b]
LSD at 0.05	0.010	0.010	0.049	0.062	0.003	0.008

Values in each column when accompanied with similar letters, were not significantly different by using the least Significant Difference at 0.05 for comparing the means.

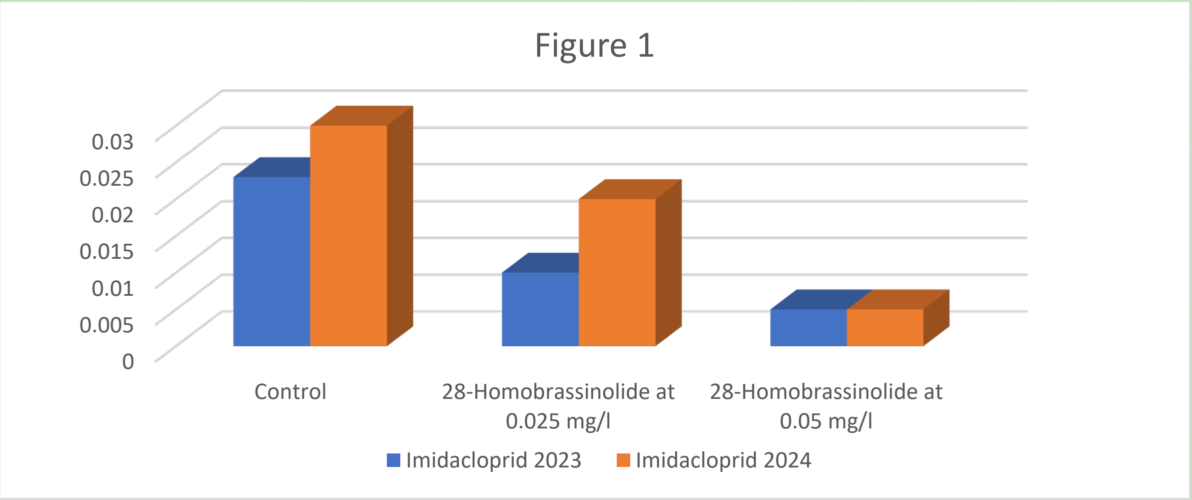

Figure1. Effect of treatments on pesticide residues (Imidacloprid) in both seasons 2023 and 2024.

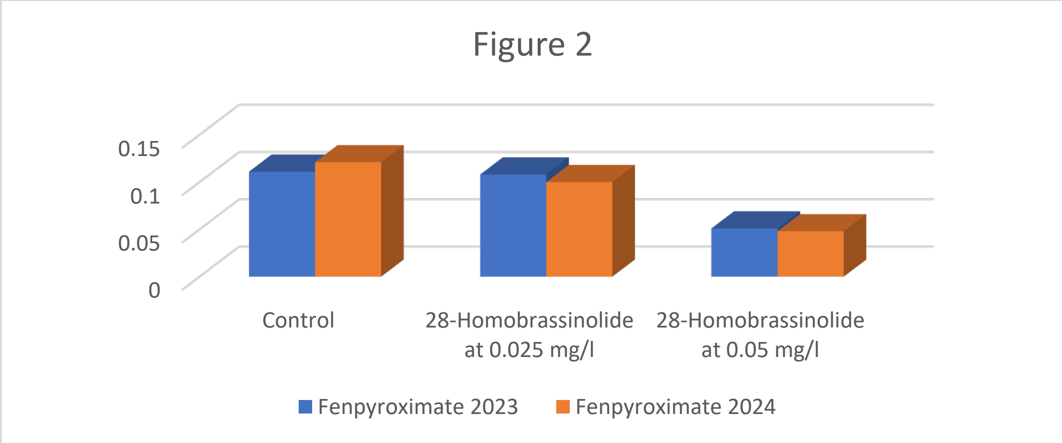

Figure 2. Effect of treatments on pesticide residues (Fenpyroximate) in both seasons 2023 and 2024.

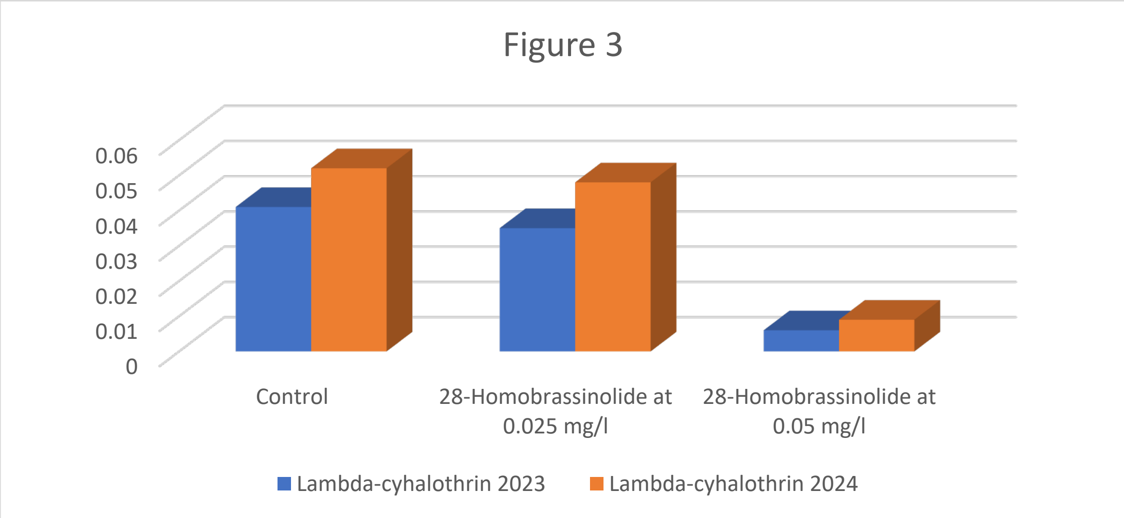

Figure 3. Effect of treatments on pesticide residues (Lambda-cyhalothrin) in both seasons 2023 and 2024.

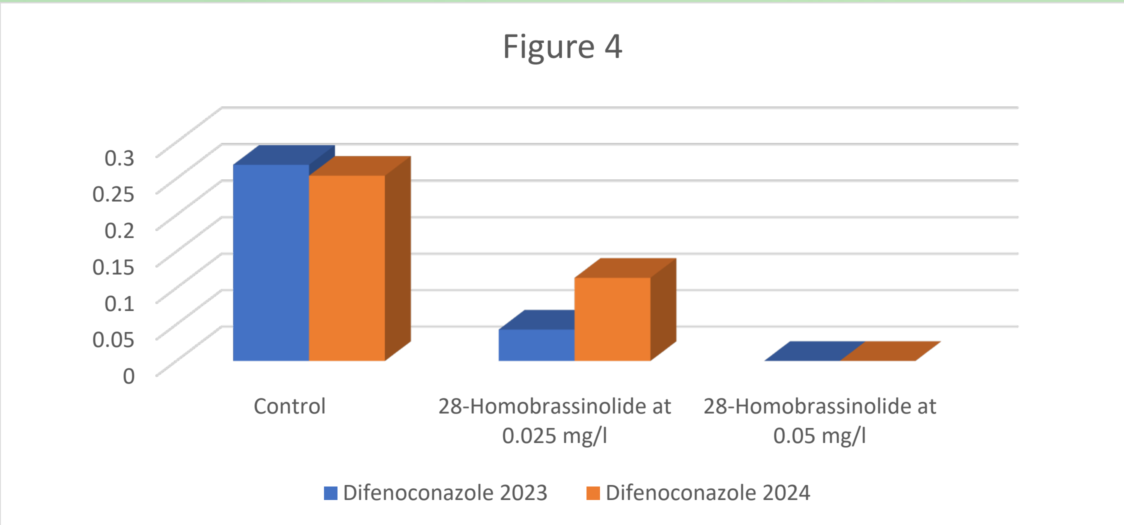

Figure 4. Effect of treatments on pesticide residues (Difenoconazole) in both seasons 2023 and 2024.

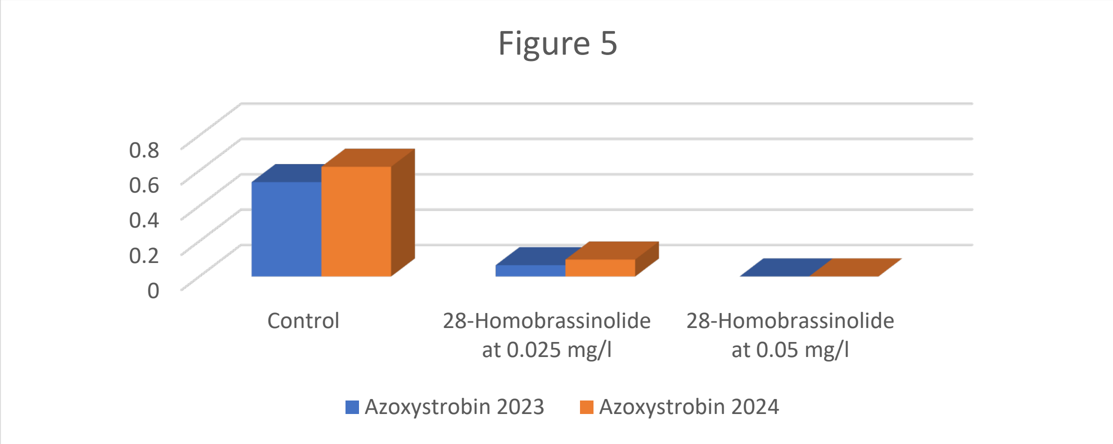

Figure 5. Effect of treatments on pesticide residues (Azoxystrobin) in both seasons 2023 and 2024.

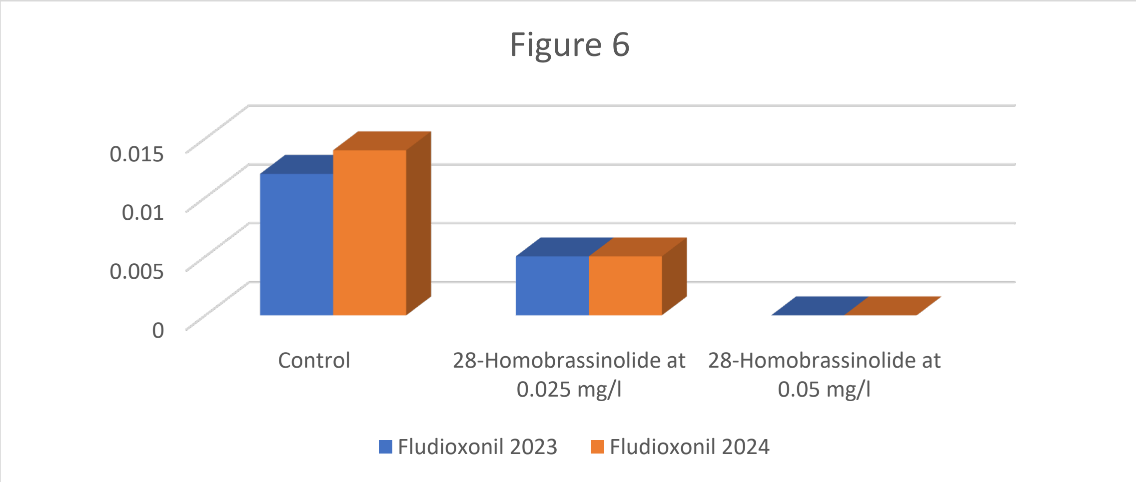

Figure 6. Effect of treatments on pesticide residues (Fludioxonil) in both seasons 2023 and 2024.

Discussion

Brassinosteroids (BRs) have a wide range of biological roles (Nolan et al., 2020; Qiao et al., 2022). 28-Homobrassinolide (HBL) is gaining attention because of its high advantages, such as increasing chlorophyll content and photosynthetic activity, accelerating translocation from source to economic parts. HBL increases the enzyme levels, which are responsible for sugars, proteins and nucleic acid. HBL promotes the proline production, which imparts a greater increase in plants' resistance against adverse weather conditions than other BRs (Sridhara et al., 2021). (Edupuganti et al., 2019) already showed improvement in maize crop growth due to exogenously applied HBL. However, extensive studies with respect to different dosages and application methods were not properly documented, which has played a key role in decreasing the cost and quantity of HBL.

These BRs play important roles in development, plant growth, adaptation to environmental stress and fruit quality (Siddiqi & Husen, 2021; Zhang & Yang, 2021; shehata, 2024). By controlling the antioxidant system, the application of exogenous BRs makes plants more resistant to the toxicity of pesticides (Sharma et al., 2018; Zhou et al., 2015). As an example, pretreatment with 24-epibrassinolide, a BR analogue, can reduce the oxidative stress response in cucumbers (Cucumis sativus) and grapes (Vitis vinifera) brought on by the insecticide chlorpyrifos and (mostly) the fungicide chlorothalonil (Wang et al., 2017; Xia et al., 2006, 2009). In mustard (Brassica juncea), castasterone, a BR, can lessen the insecticide imidacloprid's reactive toxicity (Sharma et al., 2019). Brassinosteroid signalling component (OsBZR4) plays a significant role in atrazine and isoproturon detoxification and metabolism in rice (Su et al., 2024). Yang et al., 2021 indicate that the possible risk of boscalid residue could be alleviated by EBR pretreatment through activating detoxification enzymes. This study provided further evidence about the possibility of utilizing 28-Homobrassinolide, a BR analogue, as a spray treatment to improve detoxification of pesticide residues from fresh strawberry fruits under field conditions.

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