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Research article

The effects of wheat and yam flour diets on the toxicity of three botanical extracts to *Tribolium castaneum* (Herbst) and the underlying biochemical mechanisms

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ABSTRACT

This study investigates the interactive effects of food type, exposure time, and experimental dosages on the biochemical and insecticidal responses of *Tribolium castaneum* (Herbst) to the toxic effects of three botanicals. The botanicals [*Piper guineense* (PG), *Cinnamomum verum* (CV), and *Syzygium aromaticum* (SA)] were applied individually and in binary combinations (PG + SA, PG + CV, and SA + CV). Beetles were reared for two generations on wheat and yam flour prior to insecticide exposure. The specific activities of α-amylase, alkaline phosphatase (AKP), acid phosphatase (ACP), acetylcholinesterase (AChE), and glutathione S-transferase (GST) were also determined. Generally, the insecticidal and biochemical responses of *T. castaneum* to each botanical extract and their binary combinations varied with the type of host food used for rearing the beetles, the experimental dosage, and the exposure time to the extracts. The three main factors (i.e. food, time and dosage) and their two-way interactions (F*D, F*T and D*T) significantly ($p < 0.05$) influenced the mortality response of the beetles. According to the LD_{50}, LD_{95}, and LT_{50} values, all three botanical extracts were generally more toxic to beetles fed wheat flour than those fed yam flour. Beetles given yam flour showed more toxic effects with the binary mixture of PG + CV and SA + CV than those fed wheat flour. Except for CV + SA, which had a synergistic effect on the mortality response of yam-reared *T. castaneum*, all the binary mixtures had antagonistic effects on the beetles' mortality rate. Irrespective of food type, significantly higher ($p < 0.05$) specific activities of α-amylase, AKP, and ACP was recorded in beetles exposed to CV alone and EA + CV combination relative to the control. This study demonstrates the valuable insights needed for the possible development of targeted and sustainable strategies for managing *T. castaneum* infesting wheat and yam flour.

1. Introduction

In sub-Saharan Africa and several developing nations, wheat and yam flours serve as essential mainstays in the diets of many rural and urban populations. However, their vulnerability to infestation by various insect pests continues to pose a significant threat in numerous nations. The need to safeguard yam and wheat flours from insect pest infestation remained a major challenge being encountered in large-scale flour production. Among the various insect pests that infest stored flours, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), commonly known as the red flour beetle, is noteworthy due to its potential to inflict considerable

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financial losses and threaten food security. It diminishes the quality, nutritional content, and marketability of flours [1–4]. In addition to physical damage, the infestation by *T. castaneum* markedly affects the biochemical composition of flours [5]. As a result, numerous consumers and owners of flour milling industries frequently report substantial economic losses due to the damaging actions of *T. castaneum* [6].

Consequently, the destructive activities of *T. castaneum* underline the urgent demand for rigorous and efficient pest management measures to sustain the quality and consistency of stored wheat and yam flours. The protection of stored flours from infestation by *T. castaneum* is critical in ensuring food security in many sub-Saharan countries, including Nigeria [7]. Traditional pest control methods largely rely on synthetic pesticides, raising environmental and health problems [6]. Therefore, investigating sustainable, eco-friendly pest control solutions is vital. Natural insecticides from botanicals are receiving attention as alternative pest control agents due to their various bioactive ingredients [8,9]. For instance, botanicals such as *Cinnamomum verum* (J. Presl) (Laurales: Lauraceae), *Piper guineense* (Schum and Thonn) (Piperales: Piperaceae), and *Syzygium aromaticum* (L.) Merr. & L. M. Perry (Myrtales: Myrtaceae) are notable for their medicinal and preservation capabilities.

The three botanicals (*C. verum, P. guineense*, and *S. aromaticum*) could also help in safeguarding various stored products against insect pest attack due to the presence of diverse insecticidal bioactive compounds. For example, *C. verum* contains eugenol, cinnamaldehyde, cinnamyl acetate, and camphor as their primary insecticidal chemicals [10]. Likewise, *P. guineense* contain piperine [11], whereas *S. aromaticum* has the volatile eugenol, caryophylline, and oleanol as their major insecticidal chemicals [12]. Consequently, the insecticidal activity of *P. guineense* against *Sitophilus zeamais* [13], *Callosobruchus maculatus* [14], and *Acanthoscelides obtectus* [15] have been observed and connected to the presence of piperine. The insecticidal and repellant efficacy of *C. verum* against the American cockroach, *Periplaneta americana* (L.) [16], and the maize weevil, *S. zeamais* [17], has been documented and attributed to the presence of cinnamaldehyde and eugenol in the bark extract. *Drosophila melanogaster* and *Culex pipiens* served as *in vivo* insect models to evaluate the insecticidal efficacy of *S. aromaticum* [18,19]. Consequently, these botanicals present a promising approach for sustainable pest management.

Despite numerous studies examining the toxicity of crude extracts from *C. verum, P. guineense*, and *S. aromatic*um on *T. castaneum* [11,20], there remains a paucity of information regarding the impact of wheat and yam flour diets on the main and combined toxic effects of these botanicals on *T. castaneum*. Numerous studies have ascribed the entomotoxic effects of plant-derived insecticides to various factors, including the compound's characteristics, the insects' metabolic capacity, the concentration or dosage of the active ingredient, duration of exposure, insect strain or species, ambient temperature, and host food, among others [6,21–24]. A comprehensive investigation examining the effects of host food, dosage, and exposure duration on the response of *T. castaneum* infesting yam and wheat flour to the toxic effects of single and binary mixtures of the aforementioned botanicals has yet to be thoroughly investigated.

The biochemical mechanisms underlying the insecticidal properties of these botanicals against *T. castaneum* are an intriguing area of study. Understanding *T. castaneum's* detoxifying mechanisms against the toxic effects of the aforementioned botanicals is crucial to appreciating how the botanicals impair the beetle's physiological processes, potentially impacting its insecticidal reactions. These mechanisms might involve interference with digestive enzymes or neurotransmitter pathways, leading to disruptions in enzymatic processes or metabolic pathways [25,26]. Like many insects, *T. castaneum* has evolved a highly efficient detoxification system, primarily involving enzymatic processes. Enzymes are responsible for metabolizing and detoxifying a broad range of synthetic and natural substances in many insects [26,27]. Their function goes beyond handling ingested toxins; it also includes protecting against natural enemies and detoxifying substances from the environment [28]. Hence, developing environmentally benign and long-lasting pest management strategies requires a thorough grasp of how different detoxification and digestive enzymes interact with insecticidal botanicals to modify the insecticidal response processes of *T. castaneum*.

However, there is scanty information on the effects of wheat and yam flour diets on the toxicity of the crude extract mixture of the aforementioned botanicals on some detoxifying and digestive enzymes of *T. castaneum*. We, therefore, hypothesized in this work that the toxicity of a single and binary mixture of *C. verum*, *P. guineense*, and *S. aromaticum* extracts to *T. castaneum* could be differentially influenced by the wheat and yam flour diets through changes in their metabolism and detoxification processes, thereby modulating the beetles' susceptibility to the botanical extracts. It is possible that wheat and yam flour diets might affect the physiology of *T. castaneum*, altering how the botanical extracts are metabolized. Consequently, the possible single and binary toxic effects of the three botanicals on *T. castaneum* as well as the effects of adult food on the biochemical response of *T. castaneum* were evaluated in this study.

2. Materials and methods

2.1. Collection and disinfestation of flour

Clean, dry, and non-infested wheat grains and yam tubers used for this research work were obtained from the food section of Isikan market, Akure, Ondo state. The wheat grains and yam tubers were then processed separately into fine powder using the Lister 5 HSP grinding mill machine. Thereafter, the powders were sieved through a 180 μm mesh size stainless-steel sieve (Retsch, Haan, Germany) and disinfested using the method described by Refs. [4,6,21]. Briefly, sieved flours were separately spread on a metal tray coated with aluminium foil paper and placed in a Gallenkamp oven at 60 °C for 90 min. A temperature of 60 °C for 90 min had been proved to be effective in controlling all the life stages of *T. castaneum* [29]. The flours were allowed to cool and later stored in separate labelled plastic containers covered with tight lids to prevent insect infestation prior to usage.

2.2. Insect culture

Adult *Tribolium castaneum* were sourced from the Department of Biology, Federal University of Technology, Akure (FUTA). The laboratory population of *T. castaneum*, used in this study, has been maintained on wheat and yam flour for more than five generations (4). These insects were kept in 1.65-L plastic jars containing 300 g of disinfested flour and 5 % Brewer's yeast. Each jar cover was replaced with muslin cloths to prevent escape and allow air circulation. One hundred (100) beetles were cultured separately for two generations on wheat and yam flours. The adults that emerged at the F2 generation from each food were later used for bioassay. The insect cultures in 1.65-L plastic jars were maintained inside an insect rearing cage at the ambient temperature (28 ± 2 °C), relative humidity (75 ± 5 %), and 12 h light/dark cycles. For each food, five 1.65-L plastic jars were used for insect culturing.

2.3. Collection and preparation of plant materials

Three botanicals [*Piper guineense* Schum and Thonn, *Syzygium aromaticum* Merr. & L. M. Perry, and *Cinnamomum verum* J. Presl] were bought from Oba Market, Akure. The voucher specimens of the three plant materials were authenticated and deposited at FUTA Herbarium (Herbarium No: *C. verum*: 0625; *P. guineense*: 0626; and *S. aromaticum*: 0627). Initially, the dried stem bark of *C. verum*, seeds of *P. guineense,* and flower buds of *S. aromaticum* were separately crushed using pestle and mortar. Then, using a Mascot Mixer Grinder (AN ISO 9001:2000; Titan Scales, Thane, Maharashtra, India), the semi-ground plant materials were separately processed into fine powder. Each plant powder was separately sieved (180 μm mesh) and stored in pre-labelled glass jars with air-tight lids prior to cold extraction. Ethanolic extracts were prepared by soaking 400 g of each plant powder in 1.6 L of ethanol for three days with periodic stirring with a handheld glass rod for 1 h at 24-h intervals [22]. The mixture was filtered and concentrated using a rotary evaporator, then air-dried and stored at 4 °C.

2.4. Contact toxicity bioassay of the three and their mixture against adult T. castaneum

The contact toxicity bioassay was done using the surface-film bioassay technique modified for *T. castaneum* by Ref. [30]. To facilitate this evaluation, a 5 % stock solution of each extract was prepared by diluting 1 mL of extract with 19 mL of ethanol. Thereafter, different dosages (0.5, 1.0, 1.5, 2.0, and 2.5 mL) of each botanical extract were taken from the corresponding 5 % stock solution using a micropipette (EN ISO 8655) and applied to the pre-labelled Petri dishes (diameter: 8.60 cm). Each of the aforementioned dosages equates to 8.60, 17.21, 25.82, 34.42, and 43.03 μL/cm^2, respectively, on each Petri dish. The dosages were chosen following a preliminary study to establish dosages that elicit mortality that ranges from 10 to 90 %. The area of each Petri dish was 58.11 cm^2, and this was used in estimating the actual dosages on each dish. Two controls were set up: a negative control (NC) and a solvent control (SC). For the negative control, the beetles were exposed to Petri dishes that were not treated with solvent or botanical extract. However, beetles in the solvent control group were treated with only the ethanol solvent in order to confirm if the solvent used as a diluent was toxic to the beetles. Each Petri dish was air-dried for 30 min before introducing 20 unsexed adult *T. castaneum*. Mortality was recorded at 24-h intervals for 6 days, and it was confirmed by a lack of movement upon prodding of their abdomen with a camel brush. The mortality data of the beetles was subjected to probit analysis [31] to determine the lethal dosage of the various extracts that elicit 50 % and 95 % mortality of *T. castaneum* at 72 h post-treatment. Similarly, the pre-determined LD_{95} of the three were prepared separately. Thereafter, the LD_{95} of each extract was added as follows:

LD_{95} of *P. guineense* + LD_{95} of *C. verum* (PG + CV)

LD_{95} of *P. guineense* + LD_{95} of *S. aromaticum* (PG + SA)

LD_{95} of *C. verum* + LD_{95} of *S. aromaticum* (CV + SA)

The surface film bioassay technique was repeated for each extract mixture as previously described for each spice extract. Adult mortality was also observed and recorded as previously described for each species. The LD_{50} of each binary was then computed using probit analysis, and the values were used in calculating the synergistic factor (SF) for the binary combinations. The SF was calculated using the method originally proposed by Ref. [32] and adopted by Ref. [33] with little modification.

$$\text{Symergistic Factor (SF)} = \frac{LD_{50} \text{ of A}}{LD_{50} \text{ of BM}}$$

Where:

A = Botanical extract with higher toxicity between the binary mixture.

BM = Binary mixture of two botanical extracts.

LD_{50} = Lethal dosage that will kill 50 % of the beetles.

Note: SF > 1 indicates synergism while SF < 1 indicates antagonism.

2.5. In vivo biochemical assay

2.5.1. Preparation of enzyme extract

The LD_{50} values of the extracts were used in biochemical assays. Forty adult *T. castaneum* were introduced into Petri dishes that

were dosed with LD_{50} of each spice and their binary mixture. This was done separately for beetles that emerged from each food type and incubated for 12 h. The Petri dishes were kept in the incubator for 12 h. Adults *T. castaneum* were now separately introduced into 5 g of wheat or yam flour and observed for 72 h. Each treatment was replicated four times. Twenty-five surviving adults of *T. castaneum* weighing about 55 mg after exposure were quickly kept at −4 °C for 5 h and allowed to freeze to death. Each replicate was then separately homogenised for 3 min with ice-cold buffer [25 mM potassium phosphate buffer (pH 7.2) EDTA and 1 mM 2-mercaptoethanol] using a hand-held glass homogeniser previously kept in an icebox [34]. Each homogenate from each treatment was made up to 1.5 mL in ice-cold buffer before being quickly centrifuged at 13,000 rpm for 10 min at 4 °C [38]. The resulting supernatants were carefully stored in aliquots at temperatures below −4 °C and served as an enzyme source until needed. All the supernatants were used within 3 days. The enzyme extracts were later used to determine the activities of α-amylase, alkaline and acid phosphatase, acetylcholinesterase, and glutathione S-transferase. The enzymes were selected due to the major roles they play in the metabolism and detoxification processes in many insect pests, including *T. castaneum*, feeding on starchy food like wheat and yam flour [6,22,30].

2.5.2. Determination of total protein concentration

Total protein content of *T. castaneum* homogenate was measured using Bradford's method with bovine serum albumin (BSA) as the standard [35]. A series of lower concentrations (2–10 μg/mL) were prepared in sterile test tubes by serial dilution from the 0.2 mg/mL BSA stock solution. Thereafter, 10 μL of the enzyme solution (from enzyme stock) was pipetted into clean and clearly labelled test tubes and made up to 800 μL with the appropriate volume of distilled water. To each test tube, 200 μL of Bradford reagent (pH 1.5) was added and allowed to incubate at room temperature (25 °C) for 20 min before taking the absorbance of each solution at a wavelength of 595 nm against the blank. The blank contained 800 μL of distilled water and 200 μL of Bradford reagent. The graph of absorbance against concentration was plotted, and the concentrations of the unknown protein solutions were extrapolated from the standard calibration curve. Enzyme activities were expressed in terms of μmol/min (U) and presented as specific activities (μmol/min/mg protein, i.e., U/mg protein).

2.5.3. Amylase activity assay

The α-amylase activity was determined using the dinitrosalicylic acid (DNS) method [36] adopted for *T. castaneum* by Ref. [30]. In summary, 0.5 mL of each enzyme extract (supernatant) was mixed with 1.5 mL of 2 % (w/v) potato starch solution and 1 mL of 0.05 M acetate buffer (pH 5.0) in a test tube. This reaction mixture was then allowed to incubate at 40 °C for 15 min in the incubator. Subsequently, 1 mL of the reaction mixture was transferred to a fresh tube containing 1 mL of 3,5-dinitrosalicylic acid and left in a water bath for 10 min. The optical density of the resulting colour was measured on a spectrophotometer at 520 nm. The blank sample contained buffer, substrate, DNS, and distilled water instead of enzyme. One unit of α-amylase activity was defined as the release of 1 μmol of glucose per minute per mL of enzyme under the specified assay conditions. The specific activity of amylase was expressed as units per milligram of protein. Protein concentrations were determined following the method outlined by Ref. [35], utilising BSA as a standard.

2.5.4. Alkaline phosphatase (AKP) assay

The AKP activity was determined based on the food and botanical type using the approach previously outlined by Bessey et al. [37] and adapted for *T. castaneum* by Ref. [30]. Para-nitrophenyl phosphate (pNPP) at a concentration of 0.67 M served as the substrate. Each 1.5 mL Eppendorf tube was labelled according to the matching label on stored supernatant tubes. The reaction mixture contained 0.5 mL of 1 M diethanolamine buffer with 0.5 mM magnesium chloride ($MgCl_2$) (pH 9.8) and 8.62 μL of 0.67 M pNPP, applied to each labelled tube. Subsequently, each mixture was incubated at 37 °C for 5 min. Following incubation, 20 μL of stored enzyme extract (supernatant) was added to each labelled incubated mixture and further incubated for 5 min at 37 °C. The reaction in each tube was then stopped by the addition of 0.5 mL of 1 M hydrochloric acid (HCl). The rise in absorbance was determined at 405 nm employing a spectrophotometer. The absorbance measurements suggest the liberation of p-nitrophenol, applying a molar extinction coefficient of 18,500 $M^{-1}cm^{-1}$. One unit (U) of activity refers to the quantity of enzyme necessary to create 1 μmol of product per minute within the test settings. The total protein content in each sample was measured using Bradford reagent, and specific activity was estimated in U/mg of total protein.

2.5.5. Acid phosphatase (ACP) assay

The ACP activity was assessed using a methodology similar to that used for AKP activity determination, with the exception that ACP operates optimally at an acidic pH of 4.8. A reaction mixture consisting of 0.5 mL of 50 mM sodium citrate buffer (pH 4.8), 5.5 mM p-nitrophenyl phosphate, and 20 μL of stored enzyme extracts (supernatants) obtained from yam- and wheat-reared beetles exposed to different botanical types was subjected to incubation for 30 min in a water bath at 37 °C. The reaction was stopped by the addition of 0.5 mL of sodium hydroxide (NaOH). The increase in absorbance was measured against a blank at 405 nm using a spectrophotometer with a molar extinction coefficient of 18,500 M^{-1} cm^{-1}. Total protein content was determined using the method of [35], and the specific activity of ACP was subsequently computed and expressed as U/mg of total protein.

2.5.6. Acetylcholine esterase (AChE) assay

The AChE activity of the yam- and wheat-reared beetles exposed to different botanical types was determined using Ellman's method [38]. Specifically, 10 μL of supernatant from each treatment from each food was transferred into pre-labelled wells of a microplate, each containing 100 μL of 5,5′-dithio-bis-2-nitrobenzoate (DTNB) at a concentration of 0.25 mM. These mixtures were then incubated at 35 °C for 15 min. Following the incubation period, 50 μL of acetylthiocholine iodide (ATCL) at a concentration of 0.25 nM

was added to each well as the substrate. The enzymatic reaction kinetics was continuously monitored for 10 min using a spectrophotometer set at 405 nm and 25 °C. The enzyme activity was determined based on the increase in absorbance observed after adding 10 μL of supernatant from the enzyme stock. Absorbance values were converted to concentration units using a molar extinction coefficient of 13,600 M^{-1} cm^{-1} for acetylthiocholine iodide. The specific activity was expressed as one μmole of acetylthiocholine iodide hydrolysed/min/ml/mg of protein at 25 °C (U/mg of protein) and pH 7.4.

2.5.7. Glutathione S-transferase (GST) assay

GST activity was determined spectrophotometrically following the protocol outlined by Ref. [39]. The aromatic substrate employed was 1-chloro-2,4-dinitrobenzene (CDNB). Each replicate's assay mixture was prepared in a 1 mL cuvette, comprising 30 μL of enzyme extract from stored supernatants, added to a mixture of 80 μL of 20 mM CDNB in ethanol, 110 μL of 0.1 M potassium phosphate buffer (pH 6.5), and 100 μL of 20 mM reduced glutathione (GSH) at 25 °C. A control containing a complete assay mixture without enzyme was also included. Absorbance readings were recorded at 340 nm for 3 min using a spectrophotometer. The molar extinction coefficient of the product used was 9.6×10^{-3} mM^{-1}. GST-specific activity was expressed as units per milligram of protein. Protein concentrations were determined according to Ref. [33], employing BSA as the standard.

2.6. Statistical analysis

All the data obtained on dosage mortality and biochemical bioassay were checked for normality and homoscedasticity, and no data transformation was required for analyses. Normality was checked using Shapiro–Wilk while homoscedasticity was checked using Levene's test. All data obtained from the dosage-mortality bioassay were converted into percentage mortality and subsequently analysed using one-way analysis of variance (ANOVA). Tukey's test was employed for post-hoc analysis to discern significant differences between means, with a significance level set at $\alpha = 0.05$. Data on percentage mortality and doses of each botanical extract were subjected to probit and log transformation, respectively, to determine the dose lethal to 50 % (LD_{50}) and 95 % (LD_{95}) of the beetles, as well as the time needed to achieve 50 % (LT_{50}) mortality [31]. Data on biochemical assays were also subjected to one-way ANOVA. The general linear model (GLM ANOVA) (full factorial model) was used to determine the main and interactive effects of adult food, dosage, and exposure time on the insecticidal response of *T. castaneum* to the toxic effects of the three and their binary combinations. All graphs were done using Microsoft Excel 2010. The Statistical Package for Social Sciences (SPSS) version 22 software package was used in analysing all the data.

3. Results

3.1. Main and interactive effects of food type, experimental dosage and exposure time on the response of T. castaneum to the single and binary combinations of the three botanicals

The main and interactive effects of food type (F), experimental dosage (D), and exposure time (T) on the response of *T. castaneum* to the single [CV, PG, and SA] and binary combinations [PG + SA, PG + CV, and CV + SA] are shown in Table 1. Food, dosage, and exposure time had significant effects ($P < 0.001$ in all cases) on *T. castaneum's* response to single and binary combinations of botanicals (Table 1). The two-way interaction of food with experimental dosage (F*D) significantly influenced ($P < 0.001$ in all cases except SA, with $P = 0.04$) the response of *T. castaneum* to the single and binary combinations of the botanicals. Similarly, the two-way interaction of food with exposure time (F*T) significantly affected ($P < 0.001$ in all cases except PG with $P = 0.02$) the response of the beetles to CV ($F_{5, 180} = 4.70$), PG ($F_{5, 180} = 3.94$), PG + SA ($F_{5, 180} = 4.78$), PG + CV ($F_{5, 180} = 55.80$), and CV + SA ($F_{5, 180} = 25.16$). Also, the two-way interaction of duration with exposure time (D*T) had a significant effect ($P < 0.001$) on the response of *T. castaneum* to only the

Table 1
Main and interactive effects of three factors on the response of *T. castaneum* to the single and binary combinations of three botanicals.

Factor	df	CV	PG	SA	PG + SA	PG + CV	CV + SA
Food (F)	1	F = 264.33	F = 338.33	F = 90.67	F = 323.52	F = 1188.81	F = 799.68
		P < 0.0001	P < 0.0001	P < 0.0001	P < 0.0001	P < 0.0001	P < 0.0001
Dosage (D)	4	F = 79.96	F = 66.50	F = 17.25	F = 604.30	F = 945.42	F = 523.32
		P < 0.0001	P < 0.0001	P < 0.0001	P < 0.0001	P < 0.0001	P < 0.0001
Exposure time (T)	5	F = 30.13	F = 20.66	F = 2.75	F = 294.56	F = 434.45	F = 274.70
		P < 0.0001	P < 0.0001	P = 0.020	P < 0.0001	P < 0.0001	P < 0.0001
F*D	4	F = 10.04	F = 5.51	F = 4.06	F = 25.09	F = 15.01	F = 7.124
		P < 0.0001	P < 0.0001	P = 0.004	P < 0.0001	P < 0.0001	P < 0.0001
F*T	5	F = 4.70	F = 3.94	F = 0.06	F = 4.78	F = 55.80	F = 25.16
		P < 0.0001	P = 0.002	P = 1.000	P < 0.0001	P < 0.0001	P < 0.0001
D*T	20	F = 1.34	F = 0.30	F = 0.16	F = 12.63	F = 11.63	F = 7.195
		P = 0.160	P = 0.999	P = 1.000	P < 0.0001	P < 0.0001	P < 0.0001
F*D*T	20	F = 0.44	F = 0.73	F = 0.09	F = 3.99	F = 1.49	F = 0.480
		P = 0.982	P = 0.80	P = 1.000	P < 0.0001	P < 0.090	P = 0.972
Error	180						

CV = *Cinnamomum verum*; PG = *Piper guineense*; SA = *Syzygium aromaticum*; df = degree of freedom.

binary mixtures of PG + SA ($F_{20, 180} = 12.63$), PG + CV ($F_{20, 180} = 11.63$), and CV + SA ($F_{20, 180} = 7.20$). Lastly, the three-way interactions of food with experimental dosage and exposure time (F*D*T) had a significant effect ($P < 0.001$) on the mortality response of beetles to only the binary mixture of PG + SA ($F_{20, 180} = 3.99$).

3.2. Lethal dosages (LD_{50} and LD_{95}) of single and binary mixtures of three plants extracts against T. castaneum fed wheat and yam flours

The adulticidal toxicity of the dosages of the three botanical extracts and their mixture at 72 h post-treatment is presented in Table 2. The positive slope of regression shows that mortality increased with an increase in dosages of the single and binary combinations of the plant materials, irrespective of the host food. All the $\chi 2$ values were significant ($P < 0.001$ in all cases except CV + SA with $P = 0.01$) for all the single and binary combinations. The dosages of CV lethal to 50 and 95 % of beetles fed wheat flour (LD_{50}: 26.62 μL/cm^2; LD_{95}: 122.67 μL/cm^2) were significantly lower than those of beetles fed yam flour (LD_{50}: 93.46 μL/cm^2; LD_{95}: 752.41 μL/cm^2) based on their fiducial limits. For PV and SA, lesser LD_{50} and LD_{95} values were also observed in beetles fed wheat flour relative to those fed yam flour, and this demonstrated higher insecticidal activity against wheat flour-fed beetles relative to yam flour-fed beetles. Significant differences ($P < 0.05$) were, however, observed between wheat and yam flour-fed beetles treated with PG only. For all the beetles exposed to the binary mixtures, yam flour-fed beetles had lesser LD_{50} and LD_{95} values compared to wheat flour-fed beetles, indicating an enhanced toxicity of the binary mixtures to yam flour-fed beetles. The only exception was CV + SA's LD_{95} against yam flour-fed beetles. Significant differences existed between the LD_{95} values for exposed yam and wheat flour-fed beetles for PG and SA. For PG + CV and CV + SA, significant differences existed between the LD_{50} values for yam and wheat flour-fed beetles, while their LD_{95} values for both food types were not significant. The synergistic effect was only observed for the binary mixture of CV + SA against yam flour-fed beetles. All other combinations elicited antagonistic effects, irrespective of food type.

3.3. Lethal time (LT_{50}) of the three botanical extracts and their binary mixtures against T. castaneum reared on yam and wheat flours

The lethal time of the single and binary mixtures of the botanicals needed to achieve 50 % mortality in *T. castaneum* is presented in Tables 3 and 4, respectively. Generally, LT_{50} values decreased with increasing dosages of the single (Table 3) and binary mixtures (Table 4) of the botanicals, with the lowest dosages at the highest experimental dosages of 43.02 μL/cm^2. Table 3 also shows that beetles fed wheat flour showed significantly lower LT_{50} values than those fed yam flours, especially at dosages of 25.81–43.02 μL/cm^2. At the highest experimental dosage, the least LT_{50} value (0.68 h) was evoked by PG in beetles fed wheat flour, while the highest LT_{50} value (239.56 h) was elicited by CV in yam flour-fed beetles (Table 3). For the binary mixtures, PG + CV and SA + CV showed significantly lesser LT_{50} values in beetles fed yam flour relative to those fed wheat flours at dosages of 17.2–43.02 μL/cm^2 (Table 4). However, the binary mixture of PG + SA showed significantly lower LT_{50} values in beetles fed wheat flour compared to those fed yam flour at dosages of 17.2, 25.81, and 34.42 μL/cm^2. The binary mixture of PG + CV evoked the least LT_{50} (38.79 h) in yam flour-fed beetles, while the highest LT_{50} (73.81 h) was evoked by the SA + CV binary mixture against wheat flour-fed beetles.

3.4. Effect of food type on the biochemical profile of T. castaneum treated with single and binary mixtures of the three botanicals

Overall, the biochemical response of *T. castaneum* fed with wheat and yam flour showed that the specific activities of the various digestive, detoxifying, and antioxidant enzymes varied with the food type and the botanical extract used. Generally, food type significantly influenced ($P < 0.001$) the specific activities of α-amylase, acid (AcP) and alkaline (AkP) phosphatases, acetylcholinesterase (AChE), and glutathione S-transferase assay (GST) in beetles exposed to all the single and binary combinations of the botanicals. Significantly higher ($P < 0.05$) activities of α-amylase were observed in wheat flour-fed beetles than in yam flour-fed beetles in negative controls, as well as those exposed to CV (Fig. 1). On the contrary, significantly higher ($P < 0.05$) α-amylase-specific activity was recorded in yam-flour-fed beetles exposed to only the binary mixtures of PG + CV and SA + CV than in wheat-flour-fed beetles

Table 2
Sub-lethal (LD_{50}) and lethal (LD_{95}) dosages of (μL/cm^2) of single and binary mixtures of the three plant extracts at 72 h post-treatment.

Plant material	Food Type	Regression equation	χ^2	*p*-value	LD_{50} (LFL-UFL)	LD_{95} (LFL-UFL)	SF (Effect)
CV	WF	Y = 2.48–3.53X	199.48	0.0001	26.62 (21.82–33.48)	122.67 (76.25–340.67)	
	YF	Y = 1.82–3.58X	79.02	0.0001	93.46 (62.05–254.12)	752.41 (360.68–13207.69)	
PG	WF	Y = 2.28–1.46X	470.16	0.0001	4.37 (0.01–8.75)	23.09 (14.16–65.73)	
	YF	Y = 2.28–3.02	160.03	0.0001	21.10 (17.07–25.57)	111.12 (71.68–264.67)	
SA	WF	Y = 2.34–3.55X	675.52	0.0001	32.57 (21.90–103.09)	163.87 (68.96–96871.20)	
	YF	Y = 2.24–4.53X	170.69	0.0001	105.50 (60.87–1468.58)	573.50 (171.74–255485.28)	
PG + SA	WF	Y = 3.12–5.45X	69.02	0.0001	56.45 (47.44–76.11)	190.68 (123.08–428.96)	0.08(A)
	YF	Y = 0.05–2.58X	41.09	0.0001	47.91 (44.42–52.76)	78.42 (70.15–90.95)	0.44(A)
PG + CV	WF	Y = 0.05–2.13X	44.66	0.0001	41.64 (38.90–45.30)	73.80 (66.37–84.76)	0.10(A)
	YF	Y = 0.04–1.16X	38.41	0.0001	30.08 (27.74–32.72)	72.59 (64.70–84.20)	0.71(A)
CV + SA	WF	Y = 0.06–2.42X	26.18	0.010	42.60 (40.54–45.15)	71.50 (66.21–78.52)	0.62(A)
	YF	Y = 0.04–1.25X	67.02	0.0001	33.97 (30.57–38.51	78.70 (67.18–98.66)	2.75(S)

LD_{50} = lethal dosage that kills 50 % of adult *T. castaneum*; LD_{95} = lethal dosage that kills 95 % of *T. castaneum*; CV = *Cinnamomum verum*; PG = *Piper guineense*; SA = *Syzygium aromaticum*; WF = wheat flour; YF = yam flour; SF = synergistic factor; S = synergism; A = antagonism; LFL: Lower fiducial limits; UFL: Upper fiducial limits.

Table 3
Lethal time (LT_{50}) (hour) of three plant extracts on adult *T. castaneum* reared on yam and wheat flours.

Dosage (μl/cm^2)	CV		PG		SA	
	YF	WF	YF	WF	YF	WF
8.6	109992.59	563.57 (262.95–14832.81)	308.41 (187.35–1190.48)	24.76	5444.10	1709.52 (362.91–1.269E+16)
17.2	734.68 (335.53–8250.73)	265.88 (129.07–11473358575)	129.34 (101.00–202.43)	15.29 (0.00–35.65)	1052.89 (362.25–4365596.68)	435.44
25.81	619.73 (292.97–6719.85)	66.11 (57.20–75.61)	39.23 (20.75–53.55)	2.27 (0.00–8.26)	444.27 (233.10–9915.83)	2275.13
34.42	354.36 (220.10–1030.49)	55.06 (34.93–73.75)	34.80 (24.46–43.52)	3.83 (0.00–11.86)	389.64 (259.85–889.60)	73.17
43.02	239.56 (183.25–370.34)	51.04 (31.15–68.35)	26.93 (16.30–35.77)	0.68	193.33 (124.70–1429.93)	21.31

LT_{50} = lethal time (hour) required to that kills 50 % of adult *T. castaneum*; CV = *Cinnamomum verum*; PG = *Piper guineense*; SA = *Syzygium aromaticum*; WF = wheat flour; YF = yam flour. Values in parenthesis represent 95 % lower and upper fiducial limits.

Table 4
Lethal time (LT_{50}) (hour) of the combination of the three plant extracts on adult *T. castaneum* reared on wheat and yam flours.

Dosage (μl/cm^2)	PG + SA		PG + CV		SA + CV	
	YF	WF	YF	WF	YF	WF
8.6	276.08 (205.71–570.97)	805.00 (508.67–1708.30)	184.95 (164.13–215.22)	49550.57	228.29 (187.13–305.89)	408.57 (281.89–868.49)
17.2	383.86 (256.88–895.82)	243.61 (203.53–311.07)	135.91 (123.90–151.93)	542.72 (379.69–942.17)	168.47 (143.49–210.56)	575.78 (356.52–1450.68)
25.81	342.93 (245.31–622.71)	138.82 (124.13–159.60)	88.08 (82.39–94.56)	174.98 (152.21–209.93)	101.72 (94.73–110.11)	283.10 (231.86–373.03)
34.42	134.28 (116.98–162.11)	91.83 (84.23–101.03)	57.78 (51.48–64.03)	118.49 (105.74–136.37)	60.48 (55.16–65.81)	201.56 (170.57–252.76)
43.02	72.33 (60.41–86.20)	61.43 (55.82–67.14)	38.79 (33.36–43.79)	70.58 (63.37–78.48)	44.67 (37.89–50.93)	73.81 (65.79–82.84)

LT_{50} = lethal time (hour) required to that kills 50 % of adult *T. castaneum*; CV = *Cinnamomum verum*; PG = *Piper guineense*; SA = *Syzygium aromaticum*; WF = wheat flour; YF = yam flour. Values in parenthesis represent 95 % lower and upper fiducial limits.

(Fig. 1). Similarly, the binary mixtures elicited significantly higher ($P < 0.05$) specific activity in beetles fed yam flour relative to the negative control (Fig. 1). The specific activities of AcP, AkP, and AChE in beetles exposed to the sub-lethal doses of the single and binary mixtures showed that significantly higher activities of the AcP (Fig. 2), AkP (Fig. 3), and AChE (Fig. 4) were elicited by the binary mixture of PG + CV and SA + CV in beetles fed yam flours relative to those fed wheat flours. The binary mixture of PG + SA evoked significantly higher activities of AcP in beetles fed wheat flour compared to those fed yam flours (Fig. 2). Similarly, wheat flour-fed beetles had significantly higher AkP activities in both controls and those treated with CV compared to yam flour-fed beetles (Fig. 3). All the binary mixtures evoked significantly higher GST activities in yam flour-fed beetles relative to wheat flour-fed beetles (Fig. 5). Relative to the negative control, significantly higher specific activities were elicited by the binary mixture of PG + CV and SA + CV in yam flour-fed beetles (Fig. 5).

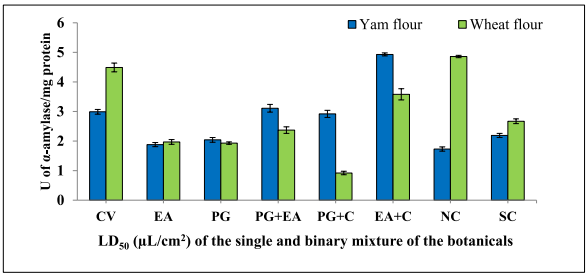

Fig. 1. Effect of food type on the specific activity of α-amylase in adult *T. castaneum* exposed to individual and binary mixture of the three botanicals.
Note: CV = *Cinnamomum verum*; PG = *Piper guineense*; SA = *Syzygium aromaticum*; NC = negative control; SC = solvent control.

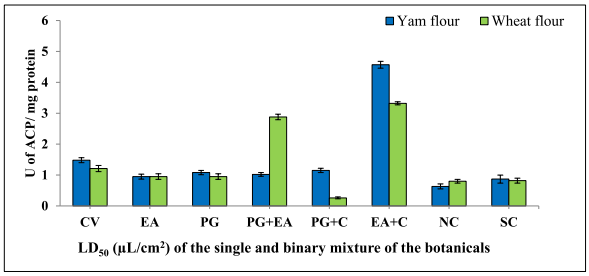

Fig. 2. Effect of food type on the specific activity of acid phosphatase in adult *T. castaneum* exposed to individual and binary mixture of the three botanicals.
Note: CV = *Cinnamomum verum*; PG = *Piper guineense*; SA = *Syzygium aromaticum*; NC = negative control; SC = solvent control.

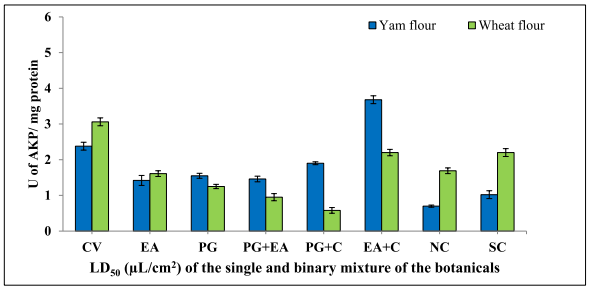

Fig. 3. Effect of food type on the specific activity of alkaline phosphatase in adult *T. castaneum* exposed to individual and binary mixture of the three botanicals.
Note: CV = *Cinnamomum verum*; PG = *Piper guineense*; SA = *Syzygium aromaticum*; NC = negative control; SC = solvent control.

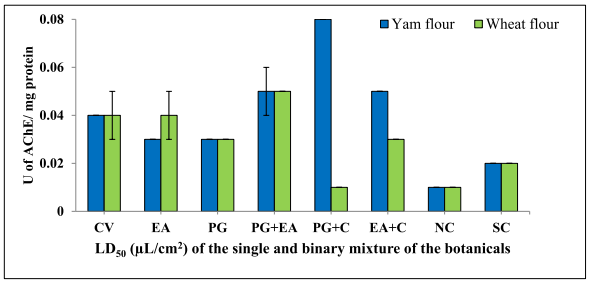

Fig. 4. Effect of food type on the specific activity of acetylcholinesterase (AChE) in adult *T. castaneum* exposed to individual and binary mixture of the three botanicals.
Note: CV = *Cinnamomum verum*; PG = *Piper guineense*; SA = *Syzygium aromaticum*; NC = negative control; SC = solvent control.

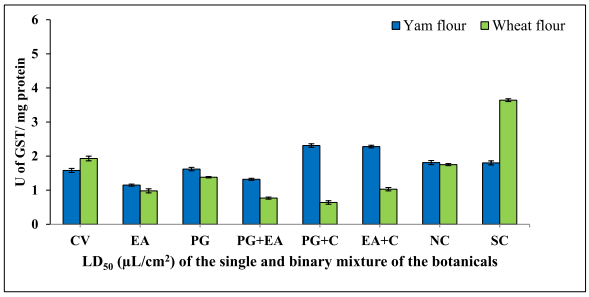

Fig. 5. Effect of food type on the specific activity of gluthione S-transferase assay (GST) in adult *T. castaneum* exposed to individual and binary mixture of the three botanicals.
Note: CV = *Cinnamomum verum*; PG = *Piper guineense*; SA = *Syzygium aromaticum*; NC = negative control; SC = solvent control.

4. Discussion

This study investigated the effects of food types on the insecticidal and biochemical responses of *T. castaneum* to single and binary mixtures of *Cinnamomum verum, Piper guineense,* and *Syzygium aromaticum*. Generally, the rate of beetle mortality depends on the type of botanical formulation, food type, exposure time, and experimental dosage. Regardless of the botanical insecticide and food type, the botanical extracts were able to evoke beetle mortality either singly or in binary combinations. This confirms their toxic effects against the red flour beetles, and it aligns with earlier research that demonstrated the efficacy of the insecticidal effects of *C. verum*, *P. guineense,* and *S. aromaticum* against *T. castaneum* [1,40–43]. The primary active compounds of the botanical oils could be responsible for the considerable contact toxicity elicited against adult *T. castaneum*. For example, the primary active compound that disrupts the cellular processes of insects in *C. verum* is cinnamaldehyde [44,45]. In *P. guineense*, the most likely active compounds responsible for its insecticidal activities are alkaloids known as piperine [46], while those of *S. aromaticum* are the volatile eugenol, caryophylline, and oleanol [47,48]. While piperine disrupts the nervous system, eugenol affects metabolic processes, leading to a more comprehensive attack on the physiology of *T. castaneum* [49,50].

Similarly, the considerable death observed in beetles exposed to the three crude oils and their binary combinations may be ascribed to the pungent smell associated with each of the extracts. It is possible that the unpleasant odour emanating from the oils and their mixture might have filled the Petri dish microenvironment and diffused through the spiracles into the respiratory system of the beetles. This could have disrupted their normal respiratory activities, leading to possible asphyxiation and subsequent death of the beetles [4]. Based on the LD_{50} values, *P. guineense* and *S. aromaticum* are the most and least toxic botanical oils, respectively, to adult *T. castaneum*, regardless of the food type used in rearing the beetles. This could be responsible for the complete beetle mortality exhibited by *P. guineense* at 72 h post-treatment, especially in wheat-reared beetles. This result can be corroborated by the findings of [42], where the toxic effects of *P. guineense* against *Callosobruchus maculatus* were established.

The combined toxic effects of botanical insecticides are usually reported by various researchers in order to reduce the use of synthetic insecticides, overcome insecticide resistance, enhance toxicity, and increase environmental safety. Overall, the binary combinations of the three botanicals did not significantly increase efficacy relative to each of the three botanical oils. Regardless of the food type, the binary mixture of *P. guineense* + *C. verum* and *P. guineense* + *S. aromaticum* had the most and least toxic effects against *T. castaneum*, respectively. Since the binary combination of black pepper and cinnamon showed lesser toxicity than either botanical alone and their synergistic factor is less than 1, the combined effect of both botanicals could thus be termed antagonistic [33]. The combination was, however, more effective with *T. castaneum* reared on wheat flour than yam flour. Similarly, all other combinations, except *S. aromaticum* with *C. verum*, showed antagonistic effects. On the contrary, the combination of black pepper and cinnamon showed a synergistic effect against *T. castaneum* reared on yam flour. This shows that the interaction between piperine and cinnamaldehyde could likely result in a multi-target approach, disrupting both the nervous and metabolic systems of *T. castaneum* more effectively than either compound alone [44]. Similarly, the combination of *S. aromaticum* and *C. verum* exhibited synergistic effects against yam-reared beetles. The synergistic effect of *S. aromaticum* with *C. verum* against yam-reared beetles could likely be due to the complementary actions of cinnamaldehyde and eugenol. Synergistic effects of plant oils have been reported in the literature, where combinations of essential oils result in enhanced insecticidal activity [41,46]. The observed results are consistent with such findings, suggesting that using combined extracts can be a more effective pest control strategy against *T. castaneum* infesting yam flour. The synergism of *S. aromaticum* with *C. verum* could help reduce the cost associated with the use of each botanical alone since a lesser quantity will be needed in the combination. The possible reduction in the total amount of botanical needed for the control of *T. castaneum* due to synergism aligns with the principles of integrated pest management (IPM) to use the least amount of pesticide necessary to achieve control. This could help reduce possible environmental contamination and the risks associated with overexposure to non-target species.

Based on the LT_{50} values, *P. guineense* still showed the highest toxicity, while *C. verum* exhibited the least toxic effects against the beetles, especially at the highest experimental concentration, irrespective of the food type. This implies that black pepper might evoke significant toxicity in the beetles that emerged from either wheat or yam flour within three days. In terms of binary combinations, *P. guineense* and *C. verum* were the most toxic to beetles fed yam flour, while *C. verum* and *S. aromaticum* had the least toxicity to beetles fed wheat flour. This demonstrated that the host food had a differential influence on the beetles' responses to single and binary combinations of botanical extracts. The effect of food type was more noticeable with the various lethal doses and lethal time values. In addition, the lethal dosage (LD_{50} and LD_{95}) and lethal time (LT_{50}) indicate that beetles fed with wheat flour were more susceptible to all three plant materials relative to those reared on yam flour. For the binary combinations, *T. castaneum* fed with wheat flour largely showed considerably higher tolerance to all the binary mixtures than those reared on yam flour. The variations in susceptibility and tolerance of the adult *T. castaneum* based on the food type could be attributed to variations in the nutritional profiles of wheat and yam flours. The differences in the nutritional composition of both flours might have affected the bioavailability and metabolism of the botanical compounds. Wheat flour, primarily composed of carbohydrates and proteins, offers a rich nutrient base that may influence how beetles react to toxins [6]. In contrast, yam flour, while also starchy, contains comparatively lower protein levels and a different micronutrient makeup, which could alter the beetle's physiological responses to insecticidal compounds [4].

The differences in mortality rates between beetles fed different flours suggest that the substrate on which pests feed has a significant impact on the efficacy of botanical insecticides. Oyeniyi et al. [22] demonstrated that the adult food influenced *T. castaneum's* nutritional physiology and insecticidal response to botanical insecticides. The various components in yam or wheat flours might have interacted with the active compounds in each botanical and their binary mixture within the gut of *T. castaneum*, resulting in reduced and enhanced tolerance to the botanical insecticides. For instance, certain proteins and fats in wheat flour might bind with botanical compounds, affecting their solubility and, subsequently, their absorption and efficacy [51]. Also, this study shows that the level of

interactions observed among the three main factors (food type, experimental dose, and exposure period) considerably influenced the response of *T. castaneum* to the botanical extracts. This demonstrated the intricate physiological mechanisms that could be influencing the response of *T. castaneum* to the individual and binary combinations of the three botanical extracts. However, the three-way interaction of food with experimental dosage and exposure time only had a significant effect on the mortality of beetles exposed to only the binary mixture of PG + SA. This showed that the degree to which food influenced the mortality of beetles was to a greater extent influenced by the experimental dosage of the binary mixture of PG + SA and time of exposure. Further, the metabolic pathways activated by different diets in *T. castaneum* could also dictate the detoxification and subsequent response to botanical compounds. Different diets can lead to the expression of varying levels of detoxification enzymes, which play crucial roles in metabolizing xenobiotics, including plant-based insecticides. A diet that induces a higher expression of these enzymes might result in reduced efficacy of the insecticides, as the beetles are better able to detoxify the compounds before they cause lethal effects. Stenberg [52] supports this perspective, having reported that the interaction between plant-based insecticides and the food types available to pests can greatly influence pest management outcomes.

The biochemical responses of *T. castaneum* considered in this study were easily influenced by the food type and the nature of sub-lethal dosages of plant materials (i.e., *C. verum*, *P. guineense*, *S. aromaticum*, and their binary combinations). In many insects, including *T. castaneum*, α-amylase is an important digestive hydrolase that enzymatically catalyses the hydrolysis of complex carbohydrates such as starch and glycogen into simpler sugars such as glucose and maltose [53,54]. Since wheat and yam flour are starchy foods, it is expected that enzymes such as α-amylase as well as acid and alkaline phosphatase will play crucial roles in the digestion of carbohydrates found in both flours. Studies have shown that α-amylase, acid, and alkaline phosphatase are mainly synthesised and secreted by the midgut epithelial cells of insects [34,55]. These enzymes are therefore critical for starchy food digestion and energy acquisition in insects, including *T. castaneum*. Both controls showed a similar trend, with significantly higher α-amylase activity in beetles fed with wheat flour compared to those fed with yam flour. This shows that the beetles fed with wheat flour produce higher levels of α-amylase relative to those fed with yam flour. This could be linked to the interaction of different components in wheat and yam flour, respectively. It is possible that higher levels of amylase in wheat-reared beetles could serve as a response to stress induced by the components in wheat flour. A similar trend was observed in beetles exposed to *C. verum*, with higher activity in beetles reared on wheat flour relative to those reared on yam flour. It is possible to suggest that the beetles are undergoing compensatory mechanisms due to the interaction of starch and other components in wheat flour with cinnamaldehyde in *C. verum* to survive the toxic effects of its sub-lethal dosage.

Irrespective of the food used in rearing *T. castaneum*, P. guineense and S. aromaticum significantly reduced α-amylase activity relative to both controls. This implies that both botanical extracts are inhibiting the α-amylase activity in the exposed beetles. This could have reduced the beetles' ability to digest starch in the flours for potential energy acquisition to defend themselves against the toxic effects of the botanicals [30]. A reduction in energy acquisition could be responsible for the deaths of the exposed beetles. For the binary combinations, higher activity was observed in beetles fed with yam flour relative to those fed with wheat flour, irrespective of the binary mixture. This indicates that the combinations might be causing more stress to beetles fed with yam flour relative to wheat flour-fed beetles, leading to an elevation of amylase activity as a general stress response [56].

The effects of the plant materials on the acid and alkaline phosphatases in beetles fed with wheat and yam flours showed that the food type influenced the beetles' responses to these enzymes. Monitoring acid and alkaline phosphatase activities in *T. castaneum* provides valuable insights into the physiological effects of the botanical insecticides considered in this study. Acid phosphatase is an enzyme that operates most effectively under acidic conditions and is responsible for breaking down phosphate esters, which are crucial for intracellular digestion and lysosomal action [57]. Alkaline phosphatase, however, functions optimally at an alkaline pH and plays an essential role in dephosphorylation processes, contributing to nutrient absorption, transport, and cellular signaling in insects [58,59]. Generally, the activities of acid phosphatase were slightly higher in beetles exposed to single and binary combinations of the insecticides relative to both controls, irrespective of the food type. This increase might signal accelerated lysosomal activity, possibly due to cellular stress or injury produced by the insecticides, resulting in an increased breakdown of cellular components [60]. This may have resulted in increased mortality in beetles exposed to these botanicals.

For alkaline phosphatase, *C. verum* and both controls showed a similar trend, with significantly increased alkaline phosphatase activities in beetles fed wheat flour compared to those fed yam flour. It could be suggested from this result that the mixture of *C. verum* with various components in wheat flour resulted in an alkaline condition in the *T. castaneum* gut, thus boosting the production of phosphate ions. This could have led to the higher increase in alkaline phosphatase activity in wheat flour-fed beetles in controls and those treated with *C. verum*. Cinnamon, however, evoked elevated alkaline phosphatase activity relative to the controls, irrespective of food type. This might be a compensatory mechanism to maintain nutrient absorption and metabolism in the presence of stress induced by *C. verum* [30]. The trend was, however, reversed with *P. guineense* and the binary combinations with higher alkaline phosphatase activity in beetles fed with yam flour relative to those fed wheat flour. All of these findings demonstrate that wheat and yam flour interact differently with insecticides to affect the activities of acid and alkaline phosphatase in *T. castaneum*.

Relative to both controls, the activities of acetylcholinesterase (AChE) were significantly increased, generally, by both the single and binary combinations of the botanicals, regardless of the food types. The only exception was observed in wheat-flour-fed beetles with the binary combination of *P. guineense* and *C. verum*. Piperine and cinnamaldehyde can interact with the nervous system, potentially increasing AChE activity as a compensatory mechanism against neurotoxic stress [27,61]. These compounds might inhibit neurotransmitter breakdown, leading to increased AChE activity. The increase in AChE activity suggests a compensatory mechanism to counteract the inhibitory effects of neurotoxic botanical compounds, as supported by Ref. [62]. The activities of glutathione S-transferase (GST) were generally reduced by most botanical treatments compared to controls, with significant increases in yam flour-fed beetles treated with *P. guineense*, *S. aromaticum,* and all three binary combinations. GST is one of the key enzymes that is

significantly implicated in insects' detoxification mechanisms due to its role in their resistance to synthetic and natural pesticides [26,27,63]. Different components, such as eugenol, caryophylline, and oleanol in *S. aromaticum*; piperine in *P. guineense*; and cinnamaldehyde in *C. verum*, may have interacted with proteins, lipids, and carbohydrates in yam flour to induce oxidative stress, leading to increased GST activities in exposed beetles. Piperine has been shown to modulate detoxification enzymes [49]. Increased GST activity in response to botanical treatments aligns with findings from Ref. [64], indicating an adaptive detoxification response. This might have increased the beetles' conversion of a significant portion of the consumed yam flour to energy needed for the detoxification of botanical insecticides [65]. On the contrary, *P. guineense, S. aromaticum*, and all the binary combinations evoked significantly fewer GST activities in beetles fed with wheat flour than the controls. This shows that the interaction of various components in wheat flour with the various compounds in the botanicals resulted in a reduction in energy production needed for the detoxification. This may have resulted in decreased GST activity compared to controls. The upsurge or decrease in GST activity in this study indicates an increase or decrease in *T. castaneum's* detoxifying ability on wheat or yam flour.

5. Conclusions

This study demonstrates the complex interactions among the various factors and how they influenced the response of *T. castaneum* to single and binary mixtures of the three botanical extracts. In this study, the three major factors (i.e., food, time, and dosage) and their two-way interactions considerably affected the response of *T. castaneum* to the toxic effects of all three botanicals and their mixture. This study showed that *T. castaneum* that infest wheat flour could be better managed using *P. guineense* than their counterpart that infest yam flour. However, beetles that infest yam flour could be better managed using a binary mixture of PG + CV and SA + CV relative to those that infest wheat flour. In addition, the present study suggests that α-amylase, as well as alkaline and acid phosphatases, play essential roles in the metabolism of wheat and yam flour diets, while acetylcholinesterase and glutathione S-transferase are crucial for the defense of *T. castaneum* against the toxic effects induced by the sublethal dosages of the botanical formulations. This study thus demonstrates the valuable insights needed for the possible development of targeted and sustainable management strategies for *T. castaneum* in homes and flour mill industries producing wheat and yam flours. Consequently, storing wheat and yam flours in close proximity in a store could present a significant risk to the control of *T. castaneum*. However, it is important to state that the various findings in this study could be easily influenced by the geographical origin of the botanical, or *T. castaneum,* and the variety of wheat grains or yam tubers used in preparing the flour diet. Studies should therefore be carried out on the impact of food type, geographical origin of insects, and various environmental factors on the biochemical and insecticidal responses of more botanical combinations.

CRediT authorship contribution statement

Emmanuel A. Oyeniyi: Writing – review & editing, Supervision, Formal analysis, Conceptualization. **Ayodeji P. Akinnuoye:** Writing – original draft, Methodology, Investigation, Data curation.

Data availability statement

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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