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Combinations of spintor with botanical powders as toxicants against red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae)

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Abstract

Postharvest insect pests constitute major threats to food security because they cause qualitative and quantitative damage to agricultural produce. Therefore, eco-friendly and cost-effective measures should be used for their management. In this study, five botanical powders (*Trema orientalis* and *Crataeva religiosa* leaves; and *Citrus tangelo*, *Citrus maxima* and *Citrus aurantifolia* peels) were admixed with Spintor® [1.25 active ingredient (a.i.) mg · kg⁻¹] and evaluated as toxicants against *Tribolium castaneum*. Each botanical powder and spintor was solely applied at 1000 mg · kg⁻¹ millet seeds. Spintor-botanical powder mixtures admixed at a ratio of 1 : 1 (w/w) were applied at 500 and 1000 mg · kg⁻¹, corresponding to 0.313 and 0.625 a. i. mg · kg⁻¹ for spintor in the mixtures, respectively. On the 14th day of exposure, the *Citrus* species admixed with spintor and applied at 500 mg · kg⁻¹ evoked significantly ($p < 0.05$) higher percentage mortality (72.22–90.28%) than what was observed in the mixture of spintor with *T. orientalis* (22.08%) or the mixture of spintor with *C. religiosa* (17.92%) applied at 500 mg · kg⁻¹. There was a significant difference ($p < 0.05$) in the Kaplan-Meier estimates of the treatments against the insects. The time required to kill 50% of the assayed insects (LT₅₀) when *Citrus* species were admixed with spintor at 500 mg · kg⁻¹ (10 days) was shorter than 14 days observed in the mixture of spintor with *T. orientalis* or *C. religiosa*. Therefore, admixing spintor with any of the *Citrus* powders [at 1 : 1 (w/w)] applied at 500 mg · kg⁻¹ seed is recommended for the protection of millet seeds against *T. castaneum*.

Keywords: botanicals, mortality, red flour beetle, Spinosad, survival analysis

Introduction

Pearl millet (*Pennisetum glaucum* L. R. Br.) ranks sixth among the cereals that are cultivated in India and parts of Africa (Khairawal *et al.* 1999; FAO 2007). Millet yields in Nigeria were predicted to reach two million metric tonnes in 2021 by Sasu (2022). The crop adapts to low fertility, drought, high temperatures, low pH, and high salinity. It can survive where wheat or maize would not survive (Izge *et al.* 2006). Its nutritional values (Mouquet-Rivier *et al.* 2008; Obadina *et al.* 2016) have been documented. It contains carbohydrates, protein, fat, vitamins B and A, calcium, iron,

zinc, potassium, and phosphorus. Oyegoke *et al.* (2012) has documented its various methods of preparation as food in Nigeria. According to Devries and Toennissen (2001) and Pattanashetti *et al.* (2016), it is often considered to be superior to maize, rice and wheat, due to its nutritional potential.

Despite the above-mentioned potential, pearl millet is usually attacked by insect pests including red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). The beetle is a serious insect pest of stored cereals (Babarinde and Adeyemo 2010;

Ali *et al.* 2021) and it is often categorized as a secondary pest (Pires *et al.* 2017; Shah *et al.* 2021; Sadiq *et al.* 2022), infesting broken or damaged grains. Furthermore, Babarinde *et al.* (2010) has reported it as a pest of plantain chips and groundnut seeds in southwestern Nigeria. Apart from its quantitative damage potential, it also secretes quinones (Sileem *et al.* 2019; Negi *et al.* 2022), which give infested flour an unpleasant odor. Large populations should prioritize bio-rational management since the exuviae of different instar larvae lower the quality of the meal. The use of botanicals (Babarinde *et al.* 2018, 2021; Aboelhadid and Youssef 2021), biological control agents (Zamani *et al.* 2013; Rehman *et al.* 2020) and application of spinosad (Andric *et al.* 2013; Babarinde *et al.* 2018) have been reported as bio-rational management practices. Adarkwah *et al.* (2017) also evaluated the mixture of botanical powder with diatomaceous earth for its control.

Using synthetic insecticides to control stored-product pests is often limited by financial constraints, environmental safety and human health hazards. These factors have led researchers to focus on the use of plant powders, extracts and oils, which are economical and eco-friendly, for the protection of crops (Damalas and Koutroubas 2020; Riyaz *et al.* 2022). Plant-derived substances have become relevant due to their effectiveness in protecting agricultural commodities, low mammalian and vertebrate toxicity, and low persistence, without any undesirable effects on animals and human beings (Kedia *et al.* 2015). The use of botanical powders has a higher chance of acceptability since its production does not involve major technical knowledge apart from drying and pulverization, which resource-poor farmers can afford. Apart from the reduced cost implications, botanical powders are compatible with other control strategies; hence, they can be used as a component of Integrated Pest Management. Incidentally, using botanical powders to protect seeds against insect pests has a major defect of low persistence because powders lose their efficacy due to their short post-application duration (Babarinde *et al.* 2008).

To improve the efficacy of botanical powders, combining them with other bio-rational products such as spinosad should be explored. Spinosad is a bacterium-based residual insecticide registered for use in more than 250 crops in over 60 countries (Hagstrum and Subramanyam 2006). It acts as a contact and stomach poison against insect pests and has low mammalian toxicity. It has been approved in the USA for use on millet and other cereals at $1 \text{ mg} \cdot \text{kg}^{-1}$. Although it has been reported to be effective against many stored-product insect pests (Subramanyam *et al.* 2012; Athanassiou and Kavallieratos 2014; Kavallieratos *et al.* 2017; Babarinde *et al.* 2018), *T. castaneum* has been reported to be less susceptible to it than other granivorous pests (Hagstrum and Subramanyam 2006). Despite

the diverse reports of its efficacy against arthropods, the tendency of target pests to build resistance against spinosad has also been reported (Li *et al.* 2016; Lira *et al.* 2020).

While much work has been done on the toxicity of spinosad applied at justifiable rates against different insects (Subramanyam *et al.* 2012; Athanassiou and Kavallieratos 2014; Kavallieratos *et al.* 2017; Wijayarathne and Rajapakse 2018), no attention has been paid to the possible interactions between spinosad and different botanical powders as grain protectants. As the combination of spinosad and botanical powders would fulfil the dual objective of cost-effectiveness and sustained efficacy, it merits empirical investigation. In the selection of botanical species for pesticide purposes, availability, affordability, ecological compatibility and ethnobotanical characteristics of the promising species should be considered. The five botanical species selected for this study [*Trema orientalis* Linn. Blume, *Crataeva religiosa* G. Forst, *Citrus tangelo* J.W. Ingrams and H.E. Moore, *C. maxima* Merr and *C. aurantifolia* (Christon)] Swingle are tropical species that are locally available with ethnobotanical characteristics that suggest their eco-friendliness. Therefore, this study was designed to evaluate the effect of admixing Spintor®, a commercial formulation of spinosad, with the selected botanical powders on their toxicity against *T. castaneum*.

Materials and Methods

Procurement and handling of experimental materials

The seeds of the Nigerian local variety of pearl millet 'Jero' (void of any insecticidal treatment) were obtained from Wazo Market, Ogbomoso, Nigeria. The seed lot was subsequently sorted to remove any exogenous materials. The five botanical powders used for this study were selected based on their ethnobotanical potential (Table 1). Fresh leaves of *T. orientalis* and *C. religiosa* were collected in Ogbomoso with the help of a traditional herbal practitioner. Authentication was done at the Botany Unit of the Department of Pure and Applied Biology, Ladoke Akintola University of Technology (LAUTECH), Ogbomoso. Fresh matured fruits of *Citrus tangelo*, *C. maxima* and *C. aurantifolia* were collected from the National Institute for Horticultural Research and Training (NIHORT), Ibadan, Nigeria, where the authentication of the *Citrus* species was done. Each leaf or fruit peel was air-dried to crispness on the laboratory bench at the Department of Crop and Environmental Protection (CEP), LAUTECH, Ogbomoso. Thereafter, each botanical sample was subsequently pulverized with the aid of mortar and pestle,

Table 1. The ethnobotanical potentials of the studied botanical species

Scientific name	Family	Common name	Ethnobotanical information	References
<i>Trema orientalis</i> Linn. Blume	Ulmaceae/ Cannabaceae	charcoal tree/ pigeon wood	as antibacterial glucose-lowering, anticonvulsive, analgesic, anti-inflammatory, anti-plasmodial activity	Rout <i>et al.</i> (2012); Rahman <i>et al.</i> (2017); Olanlokun <i>et al.</i> (2017); Oyebola <i>et al.</i> (2018)
<i>Crataeva religiosa</i> G. Forst	Capparaceae	three-leaf caper	treatment of poisonous bite	Dhivya and Kalaichelvi (2016)
<i>Citrus tangelo</i> J.W. Ingrams & H.E. Moore	Rutaceae	honeybells tangelo	treatment of fever	Paul and Cox (1995).
<i>Citrus maxima</i> Merr	Rutaceae	shaddoock/ maxima	treatment of fever	Ferguson (2002)
<i>Citrus aurantifolia</i> (Christon) Swingle	Rutaceae	lime	malaria treatment	Paul and Cox (1995)

sieved through a 212-micron sized sieve and stored in air-tight labelled plastic containers until use. Spintor® (0.125 D), a product of Dow Agroscience LLC, Indiana, USA, was obtained from an agrochemical dealer in Ibadan, Nigeria.

Insect culture

The adults of *T. castaneum* were obtained from a culture maintained in the CEP Departmental Laboratory, LAUTECH, Ogbomoso, Nigeria. A total of 50 mixed-sex adults were introduced into a 1-liter capacity glass jar with 500 g millet flour and kept under ambient conditions (temperature: $28 \pm 2^\circ\text{C}$, relative humidity: $73 \pm 2\%$, light to dark: 12 : 12 h) in four replicates. The jars were covered with plastic stoppers reinforced with 0.56 mm muslin cloth to prevent the beetles from escaping and other pests from intruding. After 14 days of infestation, the parental insects were removed from the culture, and the emerging larvae were used for subsequent maintenance of the culture.

Toxicity bioassay

The bioassay was conducted under the same laboratory conditions as described above for the insect culture. Spintor was admixed with each of the botanical powders at a ratio of 1 : 1 (w/w). The mixture was thoroughly stirred with the aid of a metallic rod to attain homogeneity and separately added to millet seeds at 10 and 20 mg per 20 g millet seeds, corresponding to 500 and 1000 mg · kg⁻¹ in Petri dishes. The application of a botanical powder-spintor mixture at 500 mg implies 250 mg botanical powder + 250 mg spintor (which implies 0.317 a.i. per kg spintor), while 1000 mg botanical powder-spintor implies 500 mg botanical powder + 500 mg spintor (which implies 0.625 a.i. per kg spintor). Each botanical powder and spintor was separately added at 1000 mg · kg⁻¹ millet seeds, while a negative control without spintor and

botanical powder was included in the experimental treatments. All experimental units of the botanical powders and spintor were carefully shaken to allow the treatments to evenly coat the seeds. Thereafter, 10 *T. castaneum* adults (<6 days old) were introduced to each treatment and arranged in a completely randomized design on a wooden shelf in the laboratory. Data on mortality were collected at the 1st, 3rd, 5th, 7th, 10th and 14th day of exposure. The inability of the assayed adults to respond to a pin probe was used as an index of mortality. The experiment was replicated three times. Where there was mortality in the negative control (untreated seeds), the observed mortality in the seeds treated with botanical powders or botanical powders admixed with spintor was corrected with Abbott's Formula (Abbott 1925).

Statistical analyses

Mortality data were subjected to analysis of variance (ANOVA) and significant means were separated using Studentized Neuman Kuells (SNK) at 5% significance level. The survival of the assayed insects and median lethal time (LT₅₀) were determined using Kaplan-Meier analysis. All analyses were done using SPSS software version 16.

Results

Regardless of the treatments, mortality progressed with the exposure period. On the 1st and 3rd days of exposure, there was no significant ($p > 0.05$) difference in the mortality of the insects in the treatments. However, on the 5th day of exposure, the mortality (44.97%), which was observed in millet seeds treated with 1000 mg/kg *C. maxima*, was significantly [$F(15,47) = 2.390$, $p = 0.0019$] higher than the 11.01–17.40% mortality observed in the sole

application of *C. tangelo*, *C. religiosa* and the mixture of *T. orientalis* or *C. religiosa* with spintor applied at 500 mg/kg. On the 7th day of exposure, the *Citrus* species admixed with spintor and applied at 500 mg · kg⁻¹ competed with the sole application of spintor (43.43%) and killed a significantly [$F(15,47) = 11.917$, $p < 0.0001$] higher percentage of assayed adults (38.73–69.03%) than what was observed in the mixture of spintor with *T. orientalis* (12.50%) or the mixture of spintor with *C. religiosa* (13.33%) applied at 500 mg · kg⁻¹. The same trend was observed on the 10th day of exposure. On the 14th day of exposure, the *Citrus* species admixed with spintor and applied at 500 mg · kg⁻¹ competed with the sole application of spintor (62.10%) and killed a significantly [$F(15,47) = 15.302$, $p < 0.0001$] higher percentage of the assayed adults (72.22–90.28%) than what was observed

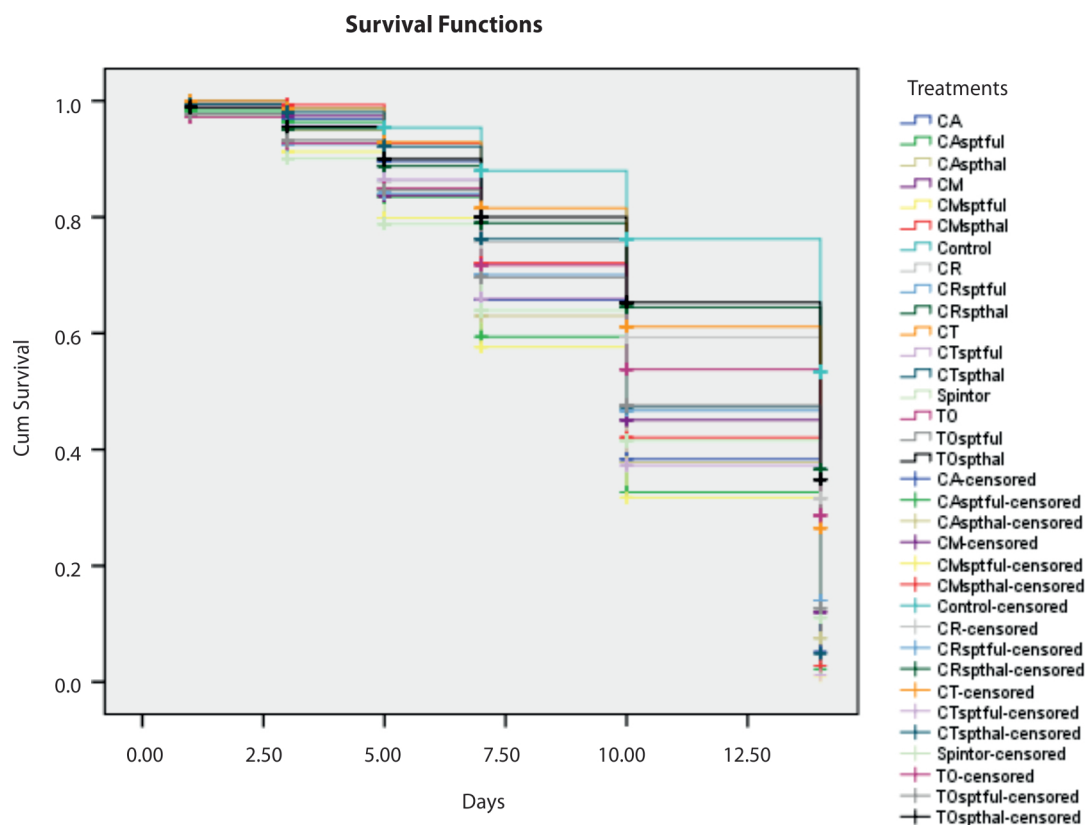
in the mixture of spintor with *T. orientalis* (22.08%) or the mixture of spintor with *C. religiosa* (17.92%) applied at 500 mg · kg⁻¹ (Table 2).

There was significant difference in the Kaplan-Meier estimates of the experimental treatments (Log Rank: df = 16, chi-square = 150.214, $p < 0.0001$), with a larger survival proportion observed in the control than the other treatments at the 7th, 10th and 14th day of exposure (Fig. 1). The results of Kaplan-Meier analysis followed the same trend as the ANOVA with a lower LT₅₀ in all the treatments having *Citrus* species admixed with spintor than what was observed in other botanical powders. *Crataeva religiosa* [14.000 (12.289–15.711) days], *C. religiosa* + spintor admixed at 500 mg · kg⁻¹ [14.000 (12.180–15.820) days], *T. orientalis* [14.00 (12.217–15.873) days], *T. orientalis* + spintor admixed at 500 mg · kg⁻¹ [14.000 (12.321–15.679)

Table 2. Percentage mortality of *Tribolium castaneum* in millet seeds treated with botanical powders and botanical powders admixed with spintor

Treatments	Rate [mg · kg ⁻¹]	% mortality on days of exposure					
		1	3	5	7	10	14
<i>Citrus tangelo</i> + spinosad	500	3.33 ± 3.33 a	10.73 ± 0.37 a	15.73 ± 4.63 ab	38.73 ± 6.18 abc	68.05 ± 3.68 de	80.74 ± 4.82 cd
<i>Citrus tangelo</i> + spinosad	1000	6.67 ± 3.33 a	3.33 ± 3.33 a	26.83 ± 3.33 ab	60.70 ± 7.44 bcd	80.55 ± 6.95 e	94.44 ± 5.56 d
<i>Citrus tangelo</i>	1000	0.00 ± 0.00 a	3.33 ± 3.33 a	11.10 ± 6.41 a	16.67 ± 11.02 a	30.55 ± 16.01 abc	36.70 ± 14.02 ab
<i>Citrus maxima</i> + spinosad	500	0.00 ± 0.00 a	3.33 ± 3.33 a	15.23 ± 3.51 ab	56.53 ± 3.62 bcd	76.39 ± 6.06 de	90.28 ± 5.01 d
<i>Citrus maxima</i> + spinosad	1000	13.33 ± 3.33 a	28.13 ± 8.73 a	41.63 ± 8.91 ab	77.97 ± 4.88 d	86.11 ± 1.39 e	94.44 ± 5.56 d
<i>Citrus maxima</i>	1000	3.33 ± 3.33 a	7.40 ± 7.40 a	49.97 ± 3.20 b	52.37 ± 7.60 bcd	54.17 ± 4.17 cde	62.10 ± 2.76 bcd
<i>Citrus aurantifolia</i> + spinosad	500	6.67 ± 3.33 a	14.43 ± 3.90 a	34.23 ± 10.66 ab	69.03 ± 5.97 bcd	69.05 ± 3.68 de	72.22 ± 2.78 cd
<i>Citrus aurantifolia</i> + spinosad	1000	10.00 ± 5.77 a	11.67 ± 9.27 a	29.62 ± 12.96 ab	82.73 ± 3.90 e	86.11 ± 1.39 e	91.07 ± 4.49 d
<i>Citrus aurantifolia</i>	1000	6.67 ± 6.67 a	7.40 ± 7.40 a	18.97 ± 7.18 ab	73.80 ± 7.32 d	77.72 ± 7.70 de	81.35 ± 3.25 cd
<i>Treva orientalis</i> + spinosad	500	6.67 ± 3.33 a	10.73 ± 0.37 a	11.10 ± 6.41 a	12.50 ± 7.22 a	17.00 ± 8.50 a	22.08 ± 11.33 a
<i>Treva orientalis</i> + spinosad	1000	13.33 ± 3.33 a	17.77 ± 3.39 a	26.37 ± 6.93 ab	38.73 ± 6.17 abc	48.61 ± 8.45 bcde	61.30 ± 6.21 bcd
<i>Treva orientalis</i>	1000	16.67 ± 3.33 a	17.40 ± 6.30 a	30.53 ± 6.98 ab	29.73 ± 10.60 ab	30.55 ± 10.02 abc	32.17 ± 8.15 ab
<i>Crataeva religiosa</i> + spinosad	500	6.67 ± 6.67 a	12.50 ± 7.22 a	13.03 ± 0.58 a	13.33 ± 3.33 a	15.27 ± 3.49 ab	17.92 ± 9.02 a
<i>Crataeva religiosa</i> + spinosad	1000	13.33 ± 3.33 a	21.10 ± 5.48 a	26.37 ± 6.93 ab	33.90 ± 10.49 ab	54.17 ± 4.17 cde	57.13 ± 7.13 bc
<i>Crataeva religiosa</i>	1000	13.33 ± 3.33 a	17.23 ± 3.91 a	17.40 ± 6.30 a	18.97 ± 3.23 ab	22.22 ± 2.78 ab	22.81 ± 7.38 a
Spintor (1.25 a.i. mg · kg ⁻¹)	1000	16.67 ± 3.33 a	31.83 ± 5.19 a	34.23 ± 10.66 ab	43.43 ± 3.62 abc	59.72 ± 5.01 cde	62.10 ± 2.76 bcd
ANOVA result (df = 15, 47)		$F = 2.326$ $p = 0.22$	$F = 2.301$ $p = 0.22$	$F = 2.390$ $p = 0.019$	$F = 11.917$ $p < 0.0001$	$F = 12.720$ $p < 0.0001$	$F = 15.302$ $p < 0.0001$

Means with the same letters within a column are not significantly different using SNK at 5% significance level



Log Rank: df = 16; chi-square = 150.214; $p < 0.0001$

Fig. 1. Kaplan-Meier Survival Curve of *Tribolium castaneum* treated with botanical powders and botanical powders admixed with spintor

CA – *Citrus aurantifolia* applied at 1000 mg/kg; CAsptful: *Citrus aurantifolia* + spintor applied at 1000 mg/kg; CAspthal: *Citrus aurantifolia* + spintor applied at 500 mg · kg⁻¹; CM – *Citrus maxima* applied at 1000 mg · kg⁻¹; CMsptful – *Citrus maxima* + spintor applied at 1000 mg · kg⁻¹; CMspthal: *Citrus maxima* + spintor applied at 500 mg · kg⁻¹; CT – *Citrus tangelo* applied at 1000 mg · kg⁻¹; CTsptful – *Citrus tangelo* + spintor applied at 1000 mg · kg⁻¹; CTspthal – *Citrus tangelo* + spintor applied at 500 mg · kg⁻¹; CR – *Crateva religiosa* applied at 1000 mg · kg⁻¹; CRsptful – *Crateva religiosa* + spintor applied at 1000 mg · kg⁻¹; CRspthal – *Crateva religiosa* + spintor applied at 500 mg · kg⁻¹; TO – *Treva orientalis* applied at 1000 mg · kg⁻¹; TOsptful – *Treva orientalis* + spintor applied at 1000 mg · kg⁻¹; TOspthal – *Treva orientalis* + spintor applied at 500 mg · kg⁻¹

days] and *C. tangelo* [14.000 (12.642–15.358) days] had higher LT₅₀ than the average duration of 10 days recorded in the other treatments (Table 3).

Discussion

In this study, the toxicity of only botanical powder or powder-spintor mixtures was exposure time-dependent. The mixture of the spintor with any of the five botanical powders showed higher toxicity against *T. castaneum* in stored millet grains than what was observed in the seeds treated with botanical powders alone. Several authors have reported the toxicity of botanical powders against *T. castaneum*. Babarinde and Ogunkeyede (2008) reported the toxicity of two Nigerian powders against the tenebrionid. Ahmad *et al.* (2019) evaluated *Allium sativum* L., *Zingiber officinale* Rosch, *Cymbopogon citratus* DC (Stapf.), *Eucalyptus globulus* Labill, *Nicotiana tabacum* L. and *Azadirachta*

indica A. Juss from Pakistan. *Allium sativum* and *Z. officinale* were more effective than other powders when admixed with rice grains, while *A. indica* admixed with wheat was also effective in controlling *T. castaneum*. Abdullahi *et al.* (2010) evaluated the toxicity of *C. sinensis* peel powder and reported dose- and exposure time-dependent toxicity at 24 hour intervals. Nta *et al.* (2017) evaluated the insecticidal potential of peels of sweet orange, tangerine, lemon, grape and lime admixed separately with popcorn grains against *T. castaneum* and reported dose-dependent efficacy. They also reported that tangerine and sweet orange performed better than other *Citrus* species.

Several authors have reported the insecticidal potentials of *Citrus* species against other stored-product insect pests besides *T. castaneum*. For instance, Don-Pedro (1985) reported the toxicity of grapefruit and orange peels against *Dermestes maculatus* (De Geer) and *Callosobruchus maculatus* (Fabricius). Recently, the repellence and toxicity of the peel powder of *C. medica* and *C. nobilis* against *C. maculatus* were investigated by

Table 3. Mean and median Survival Time (days) of *Tribolium castaneum* treated with botanical powders and botanical powders admixed with spintor

Treatments	Rate [mg · kg ⁻¹]	Mean ^a				Median (LT ₅₀)			
		estimate	std. error	95% confidence interval		estimate	std. error	95% confidence interval	
				lower bound	upper bound			lower bound	upper bound
<i>Citrus tangelo</i> + spintor	500	10.973	0.333	10.321	11.625	10	0.542	8.938	11.062
<i>Citrus tangelo</i> + spintor	1000	10.094	0.329	9.45	10.738	10	0.506	9.009	10.991
<i>Citrus tangelo</i>	1000	11.725	0.32	11.097	12.353	14	0.693	12.642	15.358
<i>Citrus maxima</i> + spintor	500	10.687	0.322	10.055	11.318	10	0.527	8.968	11.032
<i>Citrus maxima</i> + spintor	1000	9.377	0.339	8.712	10.042	10	0.515	8.991	11.009
<i>Citrus maxima</i>	1000	10.394	0.347	9.715	11.074	10	0.647	8.732	11.268
<i>Citrus aurantifolia</i> + spintor	500	9.972	0.34	9.305	10.639	10	0.574	8.875	11.125
<i>Citrus aurantifolia</i> + spintor	1000	9.653	0.331	9.004	10.301	10	0.514	8.992	11.008
<i>Citrus aurantifolia</i>	1000	10.215	0.332	9.564	10.866	10	0.545	8.932	11.068
<i>Treva orientalis</i> + spintor	500	11.704	0.334	11.05	12.358	14	0.857	12.321	15.679
<i>Treva orientalis</i> + spintor	1000	10.505	0.354	9.812	11.198	10	0.625	8.775	11.225
<i>Treva orientalis</i>	1000	10.802	0.358	10.1	11.504	14	0.91	12.217	15.783
<i>Crateva religiosa</i> + spintor	500	11.583	0.34	10.916	12.251	14	0.929	12.18	15.82
<i>Crateva religiosa</i> + spintor	1000	10.459	0.354	9.766	11.152	10	0.636	8.753	11.247
<i>Crateva religiosa</i>	1000	11.186	0.352	10.496	11.877	14	0.873	12.289	15.711
Spintor (1.25 a. i. mg/kg)		9.904	0.359	9.199	10.608	10	0.646	8.734	11.266
Untreated Control		12.569	0.286	12.009	13.129		.	.	.
Overall		10.654	0.084	10.49	10.817	10	0.155	9.696	10.304

Harshani and Karunaratne (2021). According to their results, repellence and toxicity were dose-dependent, and *C. nobilis* performed better than *C. medica*. Babarinde *et al.* (2018) recently reported that admixing spintor with *Aframomum melegueta* Schum seed powder was less effective than its combination with either *Eugenia aromatica* L. (Baill) or *Piper guineense* Schum. & Thonn. seed powder as melon seed protectants against *T. castaneum*. Also, Khorrami *et al.* (2018) reported the toxicity of botanical powders admixed with diatomaceous earth against *T. castaneum*.

The longer lethal time observed in millet seeds treated with spintor admixed with *T. orientalis* or *C. religiosa* powder at 500 mg · kg⁻¹ than what was observed in the millet seeds treated with *Citrus* powders admixed with spintor at the same dosage suggests that *T. orientalis* or *C. religiosa* had antagonistic interaction with spintor at that dosage. The lethal time values observed in this experiment are relatively higher than what was reported by previous authors who worked on *T. castaneum*. This implies that those authors had more effective botanical formulations than what is reported in the present study. For instance, Adarkwah *et al.* (2017) reported LT₅₀ of botanicals mixed with diatomaceous earth to range from 55.7 h

(2.32 days) to 62.5 h (2.6 days). Babarinde *et al.* (2014) reported LT₅₀ of *Hoslundia opposita* Vahl essential oil to be 10.24 h (0.42 day). These observations imply that the lethality of botanicals against insect pests depends on their formulations and the methodology of the bioassay. The efficacy of powder formulation usually takes effect at a longer post-application period than extracts or essential oil. This is because the bioactive compounds in the extracts and essential oils are more readily released against the target pests than in the powders. In botanical powders, bioactive compounds are locked up with other inherent inert materials. Andric *et al.* (2013) reported the toxicity of abamectin and spinosad against *T. castaneum* infesting wheat grains to be exposure period-dependent. Adult mortality has been identified as one of the bioactivities of botanical products against insect pests (Babarinde *et al.* 2014). In a recent study, the admixture of spinosad with *E. aromatica*, *A. melegueta* and *P. guineense* evoked a higher percentage mortality of the red flour beetle in melon seeds than the sole application of *A. melegueta* throughout the experimental period (Babarinde *et al.* 2018). The results obtained in this present study show that the spintor-botanical mixtures applied at 500 mg · kg⁻¹ or spintor dosage of 1000 mg · kg⁻¹

reduced the *T. castaneum* adult population more than the sole application of the botanicals at the dosage of 1000 mg · kg⁻¹.

The sole application of spintor at 1000 mg per kg implies 1.25 mg a.i. per kg spintor. Andric *et al.* (2013) reported the control of *T. castaneum* with spinosad at the dose of 5 mg a.i. per kg wheat. The present study demonstrated that admixing botanical powders with lower doses of spinosad is effective in protecting millet grains against the infestation of *T. castaneum*. The mode of action of powder formulations that kills the target arthropods involves the abrasion of the cuticle that causes desiccation (Awam *et al.* 2012) and the blockage of the spiracle by the dust particles (EPA 1997). These two mechanisms were also postulated by Babarinde *et al.* (2018) for the observed toxicity of the botanicals against *T. castaneum*. Furthermore, insects can be exposed to lethal doses of spinosad and botanical powder-spinosad mixtures via ingestion or contact. This is because the mode of action of spinosad involves the insect's nervous system at the gamma-aminobutyric acid and nicotinic acetylcholine receptor site (Khashaveh *et al.* 2011).

Conclusions

In conclusion, all the *Citrus* species admixed with spintor and applied at 500 mg · kg⁻¹ competed with spinosad applied at 1000 mg · kg⁻¹. However, the Citrus-spintor mixtures evoked a higher percentage mortality than when *T. orientalis* or *C. religiosa* was admixed with spintor and applied at 500 mg · kg⁻¹. Therefore, *Citrus* species can be admixed with spintor to protect millet seeds against the infestation of *T. castaneum*. Further research is necessary to evaluate the efficacy of duration after treatment of the botanical powders and other formulations of *Citrus* species such as essential oil to determine the bioactive compounds in each of them.

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