

SYNERGISTIC EFFECT OF SPINOSAD WITH SELECTED BOTANICAL POWDERS
AS BIORATIONAL INSECTICIDES AGAINST ADULTS OF *TRIBOLIUM*
CASTANEUM HERBST, 1797 (COLEOPTERA: TENEBRIONIDAE)

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Abstract: The synergistic effect of spinosad with three botanical (*Aframomum melegueta*, *Eugenia aromatica* and *Piper guineense*) powders as biorational insecticides against *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae) infesting melon (*Citrullus lanatus*) seeds was investigated. Treatments included sole application of each botanical powder (50 g/kg of melon seed), sole application of spinosad (SASp) (1.0 g/kg), mixture of spinosad (0.5 g/kg) + botanical powders (25 g/kg) and an untreated control. Data were collected on tenebrionid mortality rate (%) (PM) and melon seed weight loss rate in % (PWL). Phytochemical analysis of the botanicals was also carried out. At 3–14 days after treatment (DAT), PM observed in melon seeds treated with SASp (90.00–100.00%) was not significantly ($p > 0.05$) different from PM observed in melon seeds treated with spinosad + *E. aromatica* powder (86.67–100.00%) and spinosad + *P. guineense* powder (85.00–100.00%). PWL observed in melon seeds treated with spinosad + botanical powders (1.17–1.40%) was not significantly different from PWL observed in seeds treated with SASp (0.42%), but it was significantly lower than PWL (3.28%) observed in melon seeds treated with sole application of *A. melegueta* powder. *P. guineense* powder had the highest contents of alkaloids (868.33 mg/100 g), tannins (550.00 mg/100 g), phenolics (53.57 GAE/g), and steroids (740.00 mg/100 g). *E. aromatica* powder had the highest contents of flavonoids (1466.67 mg/100 g), terpenoids (1276.00 mg/100 g) and cardiac glycosides (7.33 mg/100 g), while *A. melegueta* powder had the highest content of saponins (376.67 mg/100 g). The combination of spinosad with *P. guineense* powder or *E. aromatica* powder performed better than the combination with *A. melegueta* powder and is therefore recommended as a biorational approach for the control of *T. castaneum*.

Key words: red flour beetle, spinosad, seed, synergistic effect, melon, botanical powder.

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Introduction

Melon (*Citrullus lanatus*) is an oil crop cultivated twice in a year in many developing tropical countries. After harvest and post-harvest processing, its seed coat can be removed and the oil-rich seeds can be stored until use. According to Ojeih et al. (2007), melon (egusi) seed is nutritionally rich and is characterized by the following nutrient profile: moisture (4.6%), ash (3.7%), ether extract (45.7%), crude protein (23.4%), crude fibre (12.0%) and total carbohydrate (10.6%); mineral elements: Na, K, Ca, Mg, Mn, Cu, Zn, Fe and P. Essential amino acids which include arginine, isoleucine, leucine and phenylalanine have been identified in melon. This makes it at par with other protein-rich plant foods. In some parts of southwestern Nigeria, its oil commands greater prices than groundnut oils. Despite its nutritional uses, melon seeds can be attacked by some stored product insects like *Oryzaephilus*, *Tribolium* and *Trogoderma* species. When it is stored with other arable crops in the same storage facility, the tendency of cross-infestation by major insect pests of the companion stored products is not unlikely (Babarinde et al., 2008b).

Tribolium castaneum (Herbst) has been reported to be a major secondary pest of processed or damaged stored cereal products (Lorini and Filho et al., 2007; Babarinde and Adeyemo, 2010; Stejskal et al., 2014). Besides being known as a secondary pest of cereals, it has also been reported as a pest of plantain chips and yam flour (Babarinde et al., 2010; 2013). Our recent observation in the laboratory confirms the emergence of certain *T. castaneum* strain with the ability to infest and damage intact seeds of groundnut. It is also a pest of decorticated melon seeds. The species has assumed an economic importance because infested products contain insect fragment, benzoquinones and exuviae in addition to individuals of each life stage, which renders the products less attractive to their consumers. With the new observation of *T. castaneum*'s pest status, the necessity for its control becomes more apparent. Being polyphagous and cosmopolitan, a number of synthetic insecticides have been used for successful control of the insect pest (Islam and Talukdar, 2005; Iram et al., 2013). However, some chemicals have become ineffective against the pest due to the emergence of strains that have developed resistance against the chemicals (Guedes et al., 1996; Bajracharya et al., 2016). Moreover, synthetic chemicals like organophosphates, carbamate and organochlorine have numerous negative effects on human health and non-target beneficial organisms (Islam et al., 2011). In addition, many resource-poor local farmers find the cost of chemical control of pests to be highly unaffordable.

There is therefore the need to search for biorational methods for insect pest control in postharvest crop handling. Many indigenous plants have been used for protection of stored produce against *T. castaneum* in various countries with

marked levels of efficacy (Dales, 1996; Imtiaz et al., 1999; Babarinde and Ogunkeyede, 2008; Babarinde and Adeyemo, 2010; Popović et al., 2013; Dukić et al., 2016). *P. guineense* has established pesticidal potentials against major noxious insect pests of arable crops (Babarinde et al., 2011; Ntonifor, 2011). The toxicity of *Aframomum melegueta* against the khapra beetle, *Trogoderma granarium* Everts infesting groundnut and its repellence against the maize weevil, *Sitophilus zeamais* have been reported (Babarine and Daramola, 2006; Ukeh et al., 2009). Also, several bioactivities of *Eugenia aromatica* against insect pests have been reported (Adedire et al., 2005; Ofuya et al., 2010; Oyeniyi et al., 2015). Spinosad is an insect killer that was discovered from soil in an abandoned rum distillery in 1982, produced by fermentation. It is a fast-action commercial insecticide which can be used on outdoor ornamentals, vegetables and fruit trees, to control a broad spectrum of insects (Tescari et al., 2014; Bacci et al., 2016). Spinosad is effective against a broad range of stored product insects (Vayias et al., 2009; Athanassiou et al., 2010; Hertlein et al., 2011; Subramanyam et al., 2012; Andrić et al., 2013; Kemabonta et al., 2013).

Plant products are known to have negligible effects on beneficial insects and lower environmental impacts. They are easily affordable, available and play a useful role in Integrated Pest Management (IPM) programs in developing countries. Since most developing nations suffer from the high cost of synthetic pesticides, botanical products with modest efficacy are preferred if they are readily available and less expensive than the conventional pesticides. The production of plant powders requires no skills and knowledge and their use incurs low financial expenditure. Despite the numerous advantages of the botanical powder as a grain protectant, its major shortcoming is that it loses its efficacy sooner after application than the synthetic pesticides. This subsequently affects the effectiveness of botanicals as the pest control formulation (Babarinde et al., 2008a). Secondly, despite many reported high spinosad efficacies, the cost of its sole application may be unaffordable to postharvest crop handlers and resource-poor farmers in the developing countries. Therefore, this research was designed with the following objectives: (i) to assess the synergistic effect of spinosad on the bioactivity of selected botanical powders as protectants of stored decorticated melon seeds against *Tribolium castaneum* and (ii) to evaluate the phytochemical constituents of the studied botanicals.

Materials and Methods

Experimental site

The experiment was carried out at the Crop and Environmental Protection (CEP) Departmental Laboratory, Ladoké Akintola University of Technology (LAUTECH), Ogbomoso, Nigeria.

Insect culture and experimental conditions

T. castaneum used for the study was collected from the colony originating from old poultry feed and maintained in the CEP Departmental Laboratory, LAUTECH, Ogbomosho. The emerged adults were sub-cultured in the laboratory, and the sub-culture was maintained on wheat flour in Kilner jars in the laboratory at ambient temperature ($26 \pm 3^\circ\text{C}$) and relative humidity ($65 \pm 5\%$) until new insects emerged using an earlier described method (Babarinde and Adeyemo, 2010). Bioassays were carried out under the same conditions.

Procurement and handling of experimental materials

Pesticide-free melon seeds were obtained from Wazo Market, Ogbomosho. The initial moisture content of the seeds was 3.25%. The melon seeds were sorted to ensure that only whole, intact and uninfested seeds were used. Dried fruits of *A. melegueta*, *E. aromatica* and *P. guineense* were purchased from local herb sellers at Jagun Market, Ogbomosho, Nigeria. Exogenous materials were removed, thereafter, the dried fruits were ground using an electric laboratory hammer mill. The finely ground powder was kept in a separate air tight plastic container and placed in a wooden cupboard in the laboratory until use. Spinosad manufactured by Dow Agroscience LLC was obtained from Saro Agrosciences Ltd in Lagos, Nigeria. It was well packed and sealed in a polythene bag at purchase and so kept until use.

Evaluation of the synergistic effect of spinosad with botanicals against *Tribolium castaneum*

Eight (8) treatments were prepared and separately added to 10 g of melon seeds. The corresponding values in gram of the insecticidal product (or mixture) per kilogram of melon seeds (g/kg) are shown in Table 1.

Twenty *T. castaneum* adults, one- to five-day-old, were introduced into each treatment. The experiment was set up in three replicates. Mortality data were recorded at 1, 3, 5, 7 and 14 days after treatment (DAT) and expressed as a percentage of the total number of introduced insects as follows:

$$PM = \left(\frac{NDI}{TNI} \right) \times 100 \quad (1)$$

where PM = Percentage mortality; NDI = Number of dead insects; TNI = Total number of introduced insects.

Table 1. Applied treatments and their corresponding values in g/kg.

Treatment code	Applied doses to 10 g of melon seed	Corresponding values (g/kg)
Treatment A	500 mg of <i>Piper guineense</i> powder	50 g/kg
Treatment B	500 mg of <i>Eugenia aromatica</i> powder	50 g/kg
Treatment C	500 mg of <i>Aframomum melegueta</i> powder	50 g/kg
Treatment D	5 mg of spinosad + 250 mg of <i>Piper guineense</i> powder	0.5 g/kg + 25 g/kg
Treatment E	5 mg of spinosad + 250 mg of <i>Eugenia aromatica</i> powder	0.5 g/kg + 25 g/kg
Treatment F	5 mg of spinosad + 250 mg of <i>Aframomum melegueta</i> powder	0.5 g/kg + 25 g/kg
Treatment G	10 mg spinosad (SASp)	1 g/kg
Treatment H	Untreated control	-

Five weeks after treatment, data were taken on weights of treated melon seeds. Weight loss rate in % (PWL) was estimated as:

$$WL = OW - FW \quad (2)$$

$$PWL = (WL/OW) \times 100 \quad (3)$$

where WL = Weight loss; OW = Original weight; FW = Final weight; PWL = Percentage weight loss.

Quantitative phytochemical analysis of the studied botanicals

Phytochemical analysis of each botanical powder to quantify the contents of alkaloids, flavonoids, saponins, terpenoids, tannins, phenolics, steroids, and cardiac glycosides was done according to the standard method (Marcano and Hasenawa, 1991).

Experimental design and data analysis

The experiment was laid out in a completely randomized design, replicated three times. Data were subjected to analysis of variance and significant treatment means were separated using SNK at the 5% probability level.

Results and Discussion

Effects of botanical powders and spinosad on *Tribolium castaneum* adults and weight loss rate of melon seeds

There was no mortality in the untreated melon seeds (control). At 1 DAT, 13.33% PM observed in sole application of *E. aromatica* powder was significantly ($p < 0.05$) higher than 1.67% and 0.00% observed in spinosad only (SASp) and sole

application of other botanical powders, respectively. It was observed that sole application of each botanical powder caused a lower level of PM than the PM observed when the botanical was combined with spinosad (Table 2).

Table 2. Mortality rate (%) of *Tribolium castaneum* adults exposed to spinosad-botanical powder mixtures.

Code	Treatment	Mortality rate (%)				
		1 DAT	3 DAT	5 DAT	7 DAT	14 DAT
A	<i>Piper guineense</i> powder	0.00±0.00 ^a	3.33±3.33 ^a	51.67±6.01 ^b	81.67±6.01 ^c	95.00±5.00 ^c
B	<i>Eugenia aromatica</i> powder	13.33±6.01 ^b	48.33±4.41 ^b	75.00±7.64 ^c	96.67±3.33 ^d	100.00±0.00 ^c
C	<i>Aframomum melegueta</i> powder	0.00±0.00 ^a	1.67±1.67 ^a	3.33±1.67 ^a	10.00±2.89 ^b	11.67±4.41 ^b
D	<i>Piper guineense</i> powder + spinosad	5.00±2.89 ^{ab}	85.00±7.64 ^c	100.00±0.00 ^d	100.00±0.00 ^d	100.00±0.00 ^c
E	<i>Eugenia aromatica</i> powder + spinosad	8.33±1.67 ^{ab}	86.67±1.67 ^c	95.00±2.89 ^d	100.00±0.00 ^d	100.00±0.00 ^c
F	<i>Aframomum melegueta</i> powder + spinosad	3.33±1.67 ^{ab}	8.33±1.67 ^a	60.00±10.41 ^{bc}	95.00±5.00 ^d	100.00±0.00 ^c
G	Spinosad only	1.67±1.67 ^a	90.00±5.00 ^c	100.00±0.00 ^d	100.00±0.00 ^d	100.00±0.00 ^c
H	Control	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a
ANOVA		df=7.23 F=3.481 P<0.05	df=7.23 F=113.961 P<0.05	df=7.23 F=61.594 P<0.05	df=7.23 F=178.818 P<0.05	df=7.23 F=338.286 P<0.05

DAT: Days after treatment; means (±) standard error; means along the columns with the same letter are not significantly different using the SNK test at the 5% probability level.

At 3–14 DAT, SASp caused significantly ($p<0.05$) higher PM than what was observed in sole application of *A. melegueta* powder. However, at 3–14 DAT, PM observed in melon seeds treated with SASp (90.00–100.00%) was not significantly different from PM observed in melon seeds treated with spinosad + *E. aromatica* powder (86.67–100.00%) and *P. guineense* powder mixtures (85.00–100.00%). Furthermore, at 3–5 DAT, a combination of spinosad with any of the botanical powders caused significantly higher mortality than sole application of the respective botanical powder. A similar trend was observed at 7–14 DAT for the combination of *P. guineense* or *A. melegueta* with spinosad (Table 2). The result indicates that spinosad synergistically improved the toxicity of *E. aromatica* powder at 3–5 DAT, *P. guineense* powder at 3–7 DAT and *A. melegueta* powder at 3–14 DAT against *T. castaneum*. Weight loss rate (PWL) was significantly ($p<0.05$) lower in untreated melon seeds (2.67%) and seeds treated with sole application of *A. melegueta* powder (3.28%) than the value observed in melon seeds treated with SASp (0.42%) and *E. aromatica* powder (0.71%). However, PWL observed in melon seeds treated with spinosad + botanical powder mixtures

(1.17–1.40%) was not significantly ($p>0.05$) different from PWL observed in seeds treated with SASp (0.42%) (Figure 1). This implies that spinosad exerted a synergistic effect in combination with *A. melegueta* in the prevention of weight loss due to feeding by *T. castaneum*.

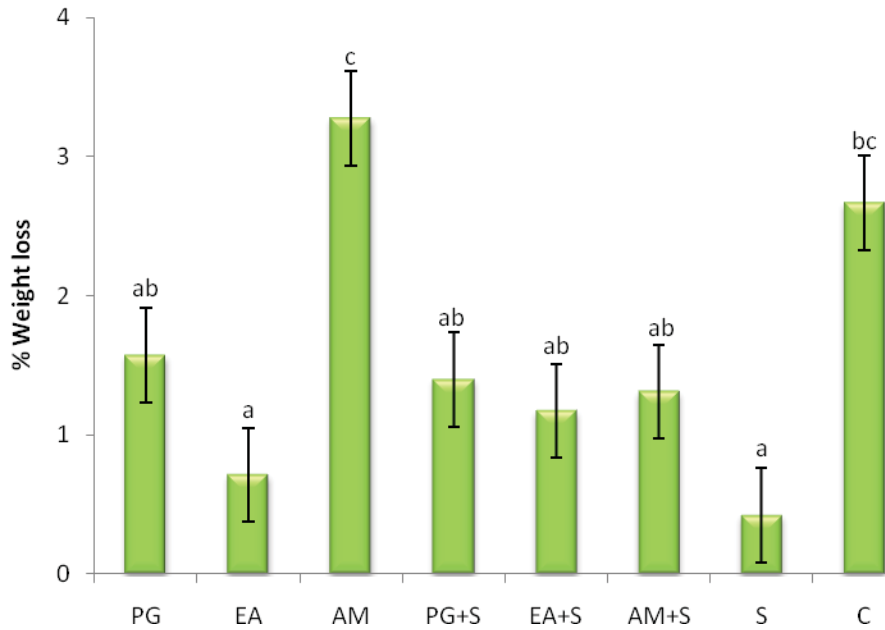


Figure 1. Weight loss rate (%) of melon seeds protected with mixtures of spinosad botanical powder due to infestation of *Tribolium castaneum* adults.

{*Piper guineense* (PG), *Eugenia aromatica* (EA), *Aframomum melegueta* (AM), *Piper guineense* + spinosad (PG+S), *Eugenia aromatica* + spinosad (EA+S), *Aframomum melegueta* + spinosad (AM+S), spinosad only (S), and untreated control (C). Means with the same letter are not significantly different using the SNK test at the 5% probability level}.

Regarding the PM data, it was observed that SASp and a mixture of spinosad with any of the 3 botanical powders showed higher efficacy in controlling *T. castaneum* infesting melon seeds compared to sole application of *A. melegueta* powder. However, all treatments caused significant PM when compared with the untreated control, where there was no mortality. At 14 DPT, 100% mortality was discovered in all treatments except that of sole application of *A. melegueta* powder. It implies that the mortality of *T. castaneum* in all spinosad botanical powder mixtures and sole application of spinosad progressed with the exposure period. A similar observation was made by Andrić et al. (2013), who evaluated the efficacy of spinosad and abamectin against different populations of *T. castaneum* infesting wheat grains. The treatments that exerted higher toxicity against *T. castaneum* also

gave better protection of melon seeds. This implies that the studied botanical insecticides had either or both adult toxicity and antifeedant effects against *T. castaneum*. PWL can be reduced when the insect population infesting the produce dies before they feed on the produce due to the toxicant present in the applied treatment. Adult mortality and antifeedant effects of botanical insecticides have been identified as noticeable mechanisms of botanical product action against insect pests (Bashir and El Shafie, 2013; Babarinde et al., 2014). Several authors have reported the modes of action of a powder formulation that kills the target arthropod. For instance, abrasion of the cuticle which consequently causes desiccation has been reported by Awam et al. (2012). Blockage of the spiracles by the dust particles has also been reported by EPA (1997).

The results on the potentials of SASp to cause adult mortality and prevent melon seed damage agree with earlier studies which reported the pesticidal potentials of spinosad against some stored product insect pests (Subramanyam et al., 2012; Nadeem et al., 2013). However, few other studies have indicated that the members of the genus *Tribolium* were least susceptible to spinosad when compared with other stored product insects (Subramanyam et al., 1999; Vayias et al., 2009). Spinosad has a low level of toxicity on non-target predatory insects. The insect pest that ingests spinosad dies within about 2 days after ingesting the active ingredient. It is eco-friendly and does not persist in the environment.

Phytochemical analysis of the studied botanical products

Table 3 shows the secondary metabolites present in the studied botanical powders. *P. guineense* powder had a significantly ($p < 0.05$) higher level of alkaloids (868.33 mg/100 g) than 771.67 and 543.33 mg/100 g present in *A. melegueta* powder and *E. aromatica* powder, respectively. Similarly, the levels of tannins (550.00 mg/100 g), phenolics (53.57 GAE/g) and steroids (740.00 mg/100 g) were significantly higher in *P. guineense* powder than the levels present in the other two botanical powders. The levels of terpenoids (1276.67 mg/100 g) and cardiac glycosides (7.33 mg/100 g) were significantly higher in *E. aromatica* powder than the levels present in the other two botanicals, whereas *A. melegueta* powder had significantly higher levels of saponins (376.67 mg/100 g) than the other two botanical powders.

Regarding the results of the phytochemical analysis of the studied botanicals, higher quantities of some secondary metabolites were found in *P. guineense* and *E. aromatica* powders than the level present in *A. melegueta* powder. For instance, the levels of alkaloids, tannins, phenols and steroids were higher in *P. guineense* powder; the levels of flavonoids and terpenoids were higher in *E. aromatica* powder, while *A. melegueta* powder had only saponins as the predominant secondary metabolites. The array of phytochemicals found in the studied botanical powder was similar to those found in those botanical products/powders elsewhere

by previous authors (Echo et al., 2012; Kadam et al., 2015). In earlier studies, the quality and quantity of phytochemicals present in botanical insecticide products were reported to be contributory to their inherent toxicity against arthropods. For instance, Rattan (2010) listed alkaloids, terpenoids and phenolics as insecticidal secondary metabolites present in many insecticidal plants.

Table 3. Phytochemical contents of the studied botanical powders.

Botanicals	Alkaloids (mg/100 g)	Flavonoids (mg/100 g)	Saponins (mg/100 g)	Terpenoids (mg/100 g)	Tannins (mg/100 g)	Phenolics (GAE/g)	Steroids (mg/100 g)	Cardiac glycosides (mg/100 g)
<i>Piper guineense</i> powder	868.33± 14.81 ^c	1065.00± 14.43 ^b	316.67± 7.26 ^b	755.00± 14.43 ^a	550.00± 7.64 ^c	53.57± 0.15 ^c	740.00± 8.66 ^c	5.33± 0.17 ^b
<i>Eugenia aromatica</i> powder	543.33± 11.67 ^a	1466.67± 13.02 ^c	266.67± 11.67 ^a	1276.67± 11.67 ^c	363.33± 7.26 ^a	45.37± 0.20 ^a	235.00± 8.66 ^a	7.33± 0.73 ^c
<i>Aframomum melegueta</i> powder	771.67± 10.93 ^b	930.00± 5.00 ^a	376.67± 7.26 ^c	830.00± 8.66 ^b	425.00± 8.66 ^b	48.57± 0.18 ^b	441.67± 11.67 ^b	2.83± 0.44 ^a
ANOVA	df=2.8 F=175.901 p<0.05	df=2.8 F=580.434 p<0.05	df=2.8 F=37.655 p<0.05	df=2.8 F=568.934 p<0.05	df=2.8 F=145.806 p<0.05	df=2.8 F=549 p<0.05	df=2.8 F=675.854 p<0.05	df=2.8 F=20.333 p<0.05

Means along the columns with the same letter are not significantly different using the SNK test at the 5% probability level.

Although the abundance of the secondary metabolites was not correlated with PM, the comparatively low level of *A. melegueta* efficacy could be due to its comparatively reduced number of the predominant secondary metabolites. Spinosad is non-persistent in the environment and can be broken down by sunlight to harmless carbon, hydrogen, oxygen and nitrogen (Saunders and Bret, 1997; Akbar et al., 2010). Hence, it is ecologically safer than some other synthetic pesticides used for protection of stored products.

Conclusion

Among the three botanical insecticides studied in this research, *P. guineense* and *E. aromatica* powders showed greater insecticidal potential than *A. melegueta* powder. A synergistic effect of spinosad was observed when combined with *P. guineense* and *E. aromatica* powders in causing the death of *T. castaneum* at 3–7 DAT. Spinosad also improved the toxicity of *A. melegueta* against *T. castaneum* and the ability of the botanical powder to reduce melon weight loss due to the infestation of *T. castaneum*. The results of this study show spinosad as a synergist for the botanical powders and establish its relevance in stored product protection. These botanicals are used in human medicine or as condiments for food and drinks

in Africa. This fact presumes their relative safety for human consumption. The dose of spinosad used in this study (0.5 g /kg mixed with botanical powders) is safe for the environment, with low toxicity against non-target organisms. Although all the botanical powders showed better ability to control *T. castaneum* when combined with spinosad than their respective sole applications, *P. guineense* and *E. aromatica* performed better than *A. melegueta*. Therefore, combination of spinosad with either *P. guineense* or *E. aromatica* powder can be an effective biorational formulation for the protection of stored melon seeds from *T. castaneum*.

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SINERGISTIČKI EFEKAT SPINOSADA I ODABRANIH BOTANIČKIH PREPARATA
 KAO BIORACIONALNIH INSEKTICIDA U SUZBIJANJU ODRASLIH JEDINKI
TRIBOLIUM CASTANEUM HERBST, 1797 (COLEOPTERA: TENEBRIONIDAE)

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R e z i m e

Ispitivan je sinergistički efekat kombinacije spinosada i tri botanička preparata dobijenih od biljaka: *Aframomum melegueta*, *Eugenia aromatica* i *Piper guineense* kao bioracionalnih insekticida u suzbijanju kestenjastog brašnara, *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae), koji napada seme lubenice (*Citrullus lanatus*). Tretmani su podrazumevali samostalnu primenu svakog botaničkog preparata (50 g/kg semena lubenice), samostalnu primenu spinosada (1,0 g/kg), smešu spinosad (0,5 g/kg) + botanički preparati (25 g/kg) i netretiranu kontrolu. Prikupljeni su podaci o stepenu mortaliteta *T. castaneum* (%) i stepenu gubitka mase semena lubenice izraženog u %. Izvršena je takođe fitohemijska analiza botaničkih preparata. U ocenama 3–14 dana posle tretiranja (DPT), stepen mortaliteta jedinki utvrđen na semenu lubenice, koje je tretirano spinosadom (90,00–100,00%) nije se značajno ($p>0,05$) razlikovao od stepena mortaliteta jedinki koji je zabeležen na semenu koje je tretirano smešom spinosad + ekstrakt biljke *E. aromatica* (86,67–100,00%) i smešom spinosad + ekstrakt biljke *P. guineense* (85,00–100,00%). Stepen gubitka mase semena koji je utvrđen na semenu lubenice koje je tretirano kombinacijom spinosad + botanički preparati (1,17–1,40%) nije se značajno razlikovao od vrednosti zabeleženih na semenu koje je tretirano samo sa spinosadom (0,42%), ali je, sa druge strane, stepen gubitka mase semena bio značajno niži u poređenju sa vrednostima utvrđenim za seme koje je tretirano samo ekstraktom *A. melegueta* (3,28%). Ekstrakt *P. guineense* imao je najviši sadržaj alkaloida (868,33 mg/100 g), tanina (550,00 mg/100 g), fenola (53,57 GAE/g), i steroida (740,00 mg/100 g). Ekstrakt *E. aromatica* imao je najviši sadržaj flavonoida (1466,67 mg/100 g), terpenoida (1276,00 mg/100 g) i glikozida (7,33 mg/100 g), dok je ekstrakt *A. melegueta* imao najviši sadržaj saponina (376,67 mg/100 g). Kombinacija spinosada i ekstrakta *P. guineense* ili *E. aromatica* se pokazala efikasnijom nego kombinacija spinosada i ekstrakta *A. melegueta*, te se stoga može preporučiti kao bioracionalna mera za suzbijanje kestenjastog brašnara, *T. castaneum*.

Ključne reči: kestenjasti brašnar, spinosad, seme, sinergistički efekat, lubenica, botanički preparat.

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