

TOXICITY OF *CHENOPODIUM AMBROSIoidES* L. (CHENOPODIACEAE) PRODUCTS FROM NIGERIA AGAINST THREE STORAGE INSECTS

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Received: April 7, 2010

Accepted: August 12, 2010

Abstract: Tests were carried out to study the toxicity, oviposition suppression, ovicidal and larvicidal effects of *Chenopodium ambrosioides* L. as powder, extracts and essential oil against *Callosobruchus maculatus* F. (Coleoptera: Bruchidae), *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) and *Tribolium castaneum* Jacqueline du Val. (Coleoptera: Tenebrionidae). The powder formulation was more toxic to *S. zeamais* than either *C. maculatus* or *T. castaneum* with 48 h LC₅₀ values of 0.46 g/kg, 1.60 g/kg and 2.14 g/kg, respectively. Ethanol extract was more toxic to *C. maculatus* with a 48 h LC₅₀ value of 0.023 g/l, than other test insect species. The essential oil treatment demonstrated higher fumigant toxicity against *C. maculatus* than *S. zeamais* with 24 h LC₅₀ values of 1.33 µl/l and 1.90 µl/l respectively. The oil vapour showed activity against *C. maculatus* egg, but had no appreciable larval mortality. The weight loss of grains admixed with *C. ambrosioides* powder was lower than the controls after 150 days of field storage.

Key words: *Chenopodium ambrosioides* L., *Callosobruchus maculatus* F., *Sitophilus zeamais* Motschulsky, *Tribolium castaneum* Jacqueline du Val., powder, extracts, essential oils

INTRODUCTION

The use of plants as insect control agents is an age old practice in Africa (Belmain and Stevenson 2001; Ewete *et al.* 2007), and *Chenopodium ambrosioides* L. (Chenopodiaceae) is a common choice. In Malawi, Southern Africa; the Congo, Central Africa, the Benin Republic, and in west Africa leaves of the shrub are dried and mixed with grains as protectant against insect infestation during storage (Maliq and Naqvi 1984; Delobel and Malonga 1987; Tapondjou *et al.* 2002). These crop protection practices are only observed in some cases as mere allegiance to tradition and not because of outstanding protectant effects (Delobel and Malonga 1987). In Nigeria *C. ambrosioides* is only used in the preparation of traditional herbal remedies against intestinal worms (Odugbemi, 2006), although recent observations showed that villagers have fewer mosquito bites when the leaves are hung on the door posts than when it is absent. It has therefore become necessary to investigate the bioactivity of the shrub against various insect groups and non-target species. The idea is to maximally exploit the potentials of the plant for insect control purposes.

Analysis of *C. ambrosioides* constituents reported by Kasali *et al.* (2006) showed that the test plant species con-

tains: sabinene (1.50%), β-pinene (0.29%), α-terpinene (55.55%), p-cymene (16.71%), limonene (1.09%), (E)-β-ocimene (0.27%), γ-terpinene (0.97%), 1,4-epoxy-p-menth-2-ene (17.72%), 1,2,3,4-diepoxy-p-menthane (0.14%) and phytol (0.38%). Except for a few reports such as Denloye *et al.* (2009) there is a scarcity of studies on the insecticidal properties of *C. ambrosioides*. Also, the few studies of the plant against storage insects are concentrated on laboratory evaluations of its toxicity to adults of test species. There is a need to establish the effect of the plant species on immature stages of storage insects and evaluate their grain protectant ability in typical traditional storage systems.

In the present study the powder, aqueous extract, ethanolic extract and essential oil of *C. ambrosioides* were tested for toxicity against the adults of three storage insects causing huge losses to grains in Africa namely: *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), *Callosobruchus maculatus* F. (Coleoptera: Bruchidae) and *Tribolium castaneum* Jacqueline du Val. (Coleoptera: Tenebrionidae). Further tests were carried out to show the effect of *C. ambrosioides* products on oviposition, egg hatching and survival of *C. maculatus* larvae. The ability of the powder to protect cowpea and maize grains from

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insect infestation in typical traditional storage systems was also evaluated.

MATERIALS AND METHODS

Preparation of formulations

Leaves of *C. ambrosioides* analyzed by Kasali *et al.* (2006) from Ipara in Badagry Local Government Area, Lagos State, Nigeria were tested for bioactivity in the powder, aqueous extract, ethanol extract and essential oil forms.

Powder and extracts

The leaf powder, aqueous and ethanol extract used in the study were prepared respectively, following the procedures already described by Denloye *et al.* (2007).

Essential oils

Essential oils were extracted from 500 g of pulverised *A. sativum*, *A. fistulosum* and *C. ambrosioides* by hydrodistillation for 2–3 h in a Clavenger apparatus (Kasali *et al.* 2006), by collecting the oil over hexane, using anhydrous sodium sulphate. The essential oils of each plant species were stored in closed glass vials at 4°C to reduce evaporative loss until when needed for bioassays.

Test Insects

Cowpea weevil – *C. maculatus*, maize weevil – *S. zeamais* Motschulsky and red flour beetle – *T. castaneum* Jacqueline du Val. from starter cultures obtained from the insectary of Nigerian Stored Product Research Institute (NSPRI), Abule-Oja, Lagos, were used as test insects. At the insectary, they were held in cultures for decades unexposed to insecticide. Fresh experimental cultures were prepared from the original stocks as described by Denloye *et al.* (2007) and maintained at 30±1°C temperature and 70±4% relative humidity.

Toxicity of powder, aqueous and ethanolic extract to adult test insect species

From preliminary dose-response bioassays six concentrations of the plant powder from 1–65 g/kg of grain were selected and tested along with the untreated controls to determine LC₅₀ values. Twenty adult *C. maculatus* (1–3-days-old) *S. zeamais* or *T. castaneum* (1–7-days-old) of mixed sexes were introduced to disinfested grains/powder admixture in 200 ml disposable plastic cups. Insect mortality was quantified after 48 h. Each treatment and control was replicated four times. Insects were counted dead when they failed to move any part of their body in response to gentle probing of the exposed abdomen with a fine brush. For the extracts, experiments which were similar to the above description were carried out, but this time 40 grains were dipped for approximately 30 secs in 0.5–8.0 g/l for the aqueous and 0.02–0.32 g/l for ethanol extract. The controls had grains dipped for approximately 30 secs in water or ethanol. All treatments and controls were replicated four times. Insects were assessed for mortality or survival after 48 h.

Rate of loss of toxicity to adult *C. maculatus* on cowpea grains

Forty undamaged cowpea grains were treated by dipping for approximately 30 secs in ethanol extracts at 0.02, 0.04, 0.08, 0.16 and 0.32 g/l, or hexane solution of essential oil at 0.50, 1.00, 2.00, 4.00 and 8.00 ml/l. In each case the dipped grains were allowed to drain on filter paper for 5 minutes before transferring into bioassay containers. For each set of treated seeds and the controls, bioassays were started off by introducing 10 unsexed 1–3-day-old adult *C. maculatus* at predetermined post-treatment time intervals of 1 h, 12 h, 24 h, 96 h, 168 h and 336 h after treatment. Each treatment, and the control treatments, were replicated four times and evaluated by assessing mortality every 24 hours for two days.

Fumigant toxicity of essential oils against adult test insects

Fumigation bioassays were carried out in air-tight Kilner jars (1 l) using the method of Don-Pedro (1996). A 7 cm – diameter Whatmann No 1 filter paper was always impregnated uniformly with the *C. ambrosioides* essential oil at 0.5, 1.00, 2.00, 4.00, 8.00 µl/l concentrations, and quickly hung down with a thread in the fumigation chamber already holding 20 adults of either *C. maculatus* or *S. zeamais* and sealed with the cap. In the control treatment insects were left in airtight sealed chambers without oil on the hung up filter paper. There were four replicates per treatment. After the 24 h fumigation period the chambers were opened and the insects assessed for mortality. Those still alive were transferred into recovery chambers for further observations.

Fumigant toxicity of *C. ambrosioides* essential oil against *C. maculatus* egg and larvae

Adult *C. maculatus* aged 0–3-days-old (2 ♂, 2 ♀) were allowed to lay eggs on cowpea seeds. Freshly laid eggs were pruned down to one per grain with the aid of binocular microscope. Twenty seeds of cowpea bearing one *C. maculatus* egg per seed were placed in the air-tight chamber and fumigated for 24 h at 2.0, 4.0, 8.0, 16.0, 32.0 µl/l concentrations with essential oil of *C. ambrosioides*. The egg bearing cowpeas were transferred after fumigation to ventilated plastic cups and after a 12 day post oviposition period, each egg was inspected for hatching under a binocular microscope.

For tests of fumigant activity of essential oil against *C. maculatus* larvae fresh cowpea seeds bearing one 6–8-day-old egg each were inspected under binocular microscope and those with hatched eggs (i.e. 1–2-day-old larvae) selected. A similar experiment as is described above was then carried out, this time 20 seeds bearing one larvae per grain were placed in the airtight chamber and fumigated for 24 h with essential oil of *Chenopodium* at 2.0, 4.0, 8.0, 16.0, 32.0 µl/l concentrations. The egg bearing cowpeas were transferred after fumigation to ventilated plastic cups and left for 21 days, after which grains were dissected and observed for mortality of larvae.

Effects of ethanol extract on oviposition and progeny development of *C. maculatus*

Cowpea seeds were treated at two concentrations (0.025 g/l and 0.10 g/l) of *C. ambrosioides* ethanolic extract by dipping for approximately 30 secs. Four 0–3-day-old adult *C. maculatus* (2 ♂, 2 ♀) were then confined for seven days to 20 treated or untreated cowpea seeds in securely covered glass Petri dishes. All treatments including the control seeds that were dipped in ethanol only, were replicated five times. At the end of the 7-day oviposition period, all adults were removed and the seeds were inspected for eggs which were counted under binocular microscope. The seeds were then kept in covered vials and monitored daily for emergent adults. Emerging adults were counted and removed from each treatment daily for 14 days after the first emergence was observed. This was done in order to prevent the overlap of generations.

Evaluation of the efficacy of *C. ambrosioides* powders as protectants of cowpea and maize grains in traditional cribs

Disinfested cowpea or maize grains (5.0 kg) were measured into jute bags and manually admixed with 2.0 g/kg of powdered *C. ambrosioides* or 0.05 g/kg of pirimiphosmethyl. Each jute bag with treated or untreated grains of each type was securely tied and stored in a traditional crib with a thatched roof in an open field for 180 days. There were four replicate bags of treated or untreated grains arranged randomly with one replicate of each treatment on each of the four layers per crib. One hundred gram samples of cowpea or maize were taken from each bag, once every 30 days and assessed for weight loss over six months.

Data analyses

Analysis of dose-response data

Analyses of toxicological dose-response data involving mortality of test insects were carried out by probit

analysis (Finney 1971), after correcting for mortality in the control using a computer programme. From these analyses median lethal concentration (LC_{50}) was derived. Analysis of variance (ANOVA) was used to compare treatment means using Statistical Package for Social Sciences (SPSS) version 11.0 (SPSS, 2001). Post-hoc analysis was carried out where there was significant difference at the 5% ($p < 0.05$) level of significance, by comparing pairs of means based on Least Significant Differences (LSD).

Monthly weight loss in each treatment and control was determined from 100 g batches of grains in each jute bag after Odeyemi and Daramola (2000) as follows:

$$\text{Percent weight loss} = \frac{(W_u \times N_d) - (W_d \times N_u) \times 100}{W_u(N_d + N_u)}$$

where:

W_u – weight of undamaged grains

N_u – number of undamaged grains

W_d – weight of damaged grains

N_d – number of damaged grains

RESULTS

Acute toxicity of *C. ambrosioides* against test insect species

The powder, aqueous extract, and ethanolic extract of *C. ambrosioides* were toxic to *C. maculatus*, *S. zeamais* and *T. castaneum*. The computed 48 h LC_{50} values showed that the powder was significantly ($p > 0.05$; no overlap in 95% confidence limits) more toxic to adult *C. maculatus* (0.05 g/kg) than either *S. zeamais* or *T. castaneum* (2.57 g/kg) (Table 1). The aqueous extract, with significantly higher 48 h LC_{50} values (without overlap in the 95% confidence limits) was less toxic than the ethanol extract. Furthermore, the ethanol extract gave comparable insecticidal activity against all the test insect species as indicated in the insignificant differences in their 48 hr LC_{50} values (Table 1).

Table 1. Toxicity of *C. ambrosioides* powder and extracts against test insect species

Formulation	Test insect species	48 h LC_{50}	95% confidence limits	Regression equation	DF	Slope [\pm SE]
Powder	<i>C. maculatus</i>	0.050 [g/kg]	0.01–0.23	$Y = 2.77 + 2.12x$	4	2.12 \pm 0.75
	<i>S. zeamais</i>	0.49 [g/kg]	0.17–1.20	$Y = 0.33 + 1.04x$	4	1.04 \pm 0.03
	<i>T. castaneum</i>	2.57 [g/kg]	1.80–3.58	$Y = -0.52 + 1.25x$	4	1.25 \pm 0.02
Aqueous	<i>C. maculatus</i>	1.21 [g/l]	0.93–1.52	$Y = -0.11 + 1.36x$	3	1.36 \pm 0.03
	<i>S. zeamais</i>	2.43 [g/l]	1.09–3.18	$Y = -0.46 + 1.19x$	3	1.19 \pm 0.03
	<i>T. castaneum</i>	2.14 [g/l]	1.67–2.77	$Y = 0.84 + 1.17x$	3	1.19 \pm 0.03
Ethanol	<i>C. maculatus</i>	0.02 [g/l]	0.02–0.03	$Y = 2.84 + 1.73x$	3	1.73 \pm 0.04
	<i>S. zeamais</i>	0.04 [g/l]	0.03–0.06	$Y = 1.99 + 1.46x$	3	1.46 \pm 0.02
	<i>T. castaneum</i>	0.04 [g/l]	0.03–0.06	$Y = 1.99 + 1.46x$	3	1.17 \pm 0.02

DF – degree of freedom

SE – standard error

LC_{50} values of with no overlap in their 95% confidence limits are not significantly different ($p < 0.05$)

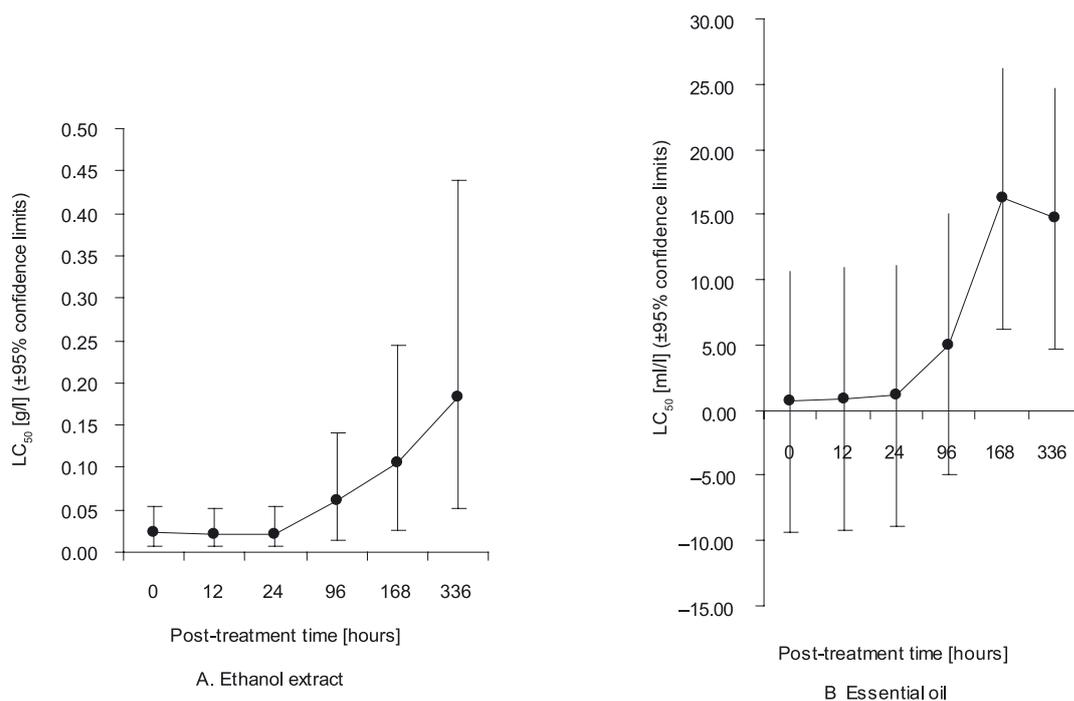


Fig. 1. Loss of toxicity of *C. ambrosioides* ethanol extract and essential oil in treated cowpea seeds

Rate of loss of toxicity of *C. ambrosioides* against *C. maculatus*

The toxicity of the ethanol extract was lost after 24 h of treating the seeds with either ethanol extract (Fig. 1a.) or essential oil (Fig. 1b.) of *C. ambrosioides* as indicated by sharp increases in LC₅₀ values.

Fumigant toxicity of *C. ambrosioides* essential oil against *C. maculatus*

Tests carried out to evaluate the fumigant action of the essential oil showed that *C. ambrosioides* oil vapour was toxic to the adult and eggs, but with no appreciable toxic effects on the larvae which are usually embedded in the grains (Table 2). The *C. ambrosioides* vapour was more

toxic to *C. ambrosioides* adult than *S. zeamais*, but demonstrated lower toxicity against either the adults or eggs of *C. maculatus*.

Effect of *C. ambrosioides* on oviposition and progeny development by *C. maculatus*

Studies on the effect of the *C. ambrosioides* ethanol extract on oviposition and adult emergence showed that seed treatment before insect introduction reduced the mean number of eggs laid from 96.00 in the control to 21.00 in the 0.10 g/l treatment. It had no effect, however, on the per cent of adults that emerged (Table 3) as a function of the number of eggs laid.

Table 2. Fumigant toxicity of essential oil to adult, egg, and larval test insect species

Test insect species		24 h LC ₅₀ [μl/l]	95% confidence limits	Regression equation	DF	Slope [±SE]
<i>C. maculatus</i>	adult	1.33	1.13–1.56	$Y = -0.26 + 2.08x$	3	2.078±0.04
	egg	2.07	1.64–2.56	$Y = -0.79 + 2.50x$	3	2.50±0.10
	larvae	43.68	30.39–53.59	$Y = -3.01 + 1.84x$	3	1.84±0.07
<i>S. zeamais</i>	adult	1.90	1.62–2.24	$Y = -0.557 + 2.00x$	3	2.00±0.034

DF – degree of freedom

SE – standard error

Table 3. Effects of Ethanol extract on oviposition and progeny production of *C. maculatus*

Treatment [g/l]	Mean number of eggs laid [±SE]	Mean adult emergence [±SE]	Mean percent adult emergence [±SE]
[0.00]	96.00±10.10 a	40.00±2.16	42.20±6.99
[0.025]	70.00±2.58 b	28.00±4.32	39.94±5.33
[0.10]	21.00±2.45 c	8.00±1.83	39.13±12.68

Column means bearing same superscripts are not significantly different ($p > 0.05$) by Least Significant Difference (LSD) test

SE – standard error

Protectant ability of *C. ambrosioides* on stored cowpea and maize grains

Both maize and cowpea were protected for up to six months in the open-field traditional storage crib. The weight loss in cowpea seeds treated with *C. ambrosioides* powder showed that significant damage to seeds oc-

curred after 120 days of storage and increased in both cowpea and maize for six months which is when the experiment was terminated. The pirimiphos-methyl significantly prevented weight loss to grains for six months relative to the control (Table 4).

Table 4. Weight loss in grains treated with *C. ambrosioides* powder or pirimiphosmethyl

Weight loss [g]							
Post-treatment time [days]							
Treatments	0	30	60	90	120	150	180
Untreated control maize	0.00	0.00	0.02±0.002 a	1.44±0.35 a	1.80±0.35 a	2.47±0.29 a	3.12±0.44
Cowpea	0.00	0.00	0.03±0.00 a	1.79±0.23 a	2.21±0.42 a	2.83±0.19 a	5.04±1.15 a
<i>C. ambrosioides</i> maize	0.00	0.00	0.0 a	0.0±0.00 b	0.02±0.00 b	0.79±0.38 c	1.80±0.41 a, c
Cowpea	0.00	0.00	0.0 a	0.0 b	0.10±0.03 b	1.09±0.78 c	2.57±0.46 c
Pirimiphosmethyl maize	0.00	0.00	0.0 a	0.0 b	0.0	0.02±0.03 b	0.03±0.03 b
Cowpea	0.00	0.00	0.0 a	0.0 b	0.0 b	0.0 b	0.05±0.01 b

Each datum is a mean of four replications; values of grain damage bearing the same letters are not significantly different by LSD ($p < 0.05$) following ANOVA; data were the square root transformed before ANOVA. Figures in parentheses are the square root transformed values

DISCUSSION

On the basis of properties required in chemicals for controlling insects that feed on grains such as: 1 – toxicity to adults; 2 – reduction of oviposition; 3 – ovicidal activity; 4 – toxicity to immature stages *C. ambrosioides*, showed some appreciable potential under these parameters. The potential of *C. ambrosioides* is in respect to activity levels measured in the present study. These findings agree with the reports of the toxicity of *C. ambrosioides* leaf powder and essential oil to *C. maculatus* thus justifying their use by farmers in Malawi and Benin Republic to protect cowpea (Delobel and Malonga 1987; Tapondjou *et al.* 2002). Relying on these results, *C. ambrosioides* may be used by subsistence farmers to protect stored cowpea against *C. maculatus* in small storage systems.

In this study, the *C. ambrosioides* product was applied in four formulations namely: powder, aqueous extract, ethanolic extract and essential oil. It is well established that the bioactivity of plant extracts depends on the solubility of their constituents in the solvent used for extraction (Marjorie 1999). The aqueous and ethanolic extracts of *C. ambrosioides* used in this study showed differential toxicities against the test storage insects. The ethanolic extracts were consistently more toxic than the aqueous extracts. Such differences in the toxicity of plant extracts obtained using different solvents as in this study were reported by earlier workers (Makanjuola 1989; Akinyemi *et al.* 2000; Doughari and Manzara 2008). The study by Makanjuola (1989), demonstrated that the leaf aqueous extract of *Azadirachta indica* was more toxic to *C. maculatus*, *S. zeamais* and *Cylas ponticolis* than the methylated spirit extracts. On the other hand a study of the antimicrobial activity of crude leaf extracts of *Mangifera indica* by Doughari and Manzara (2008) showed that the methanolic extract was more toxic to the test bacteria than the aqueous extracts. It is noteworthy that the bioactive constituents of plants arise from secondary metabolites, and in terms of their partition coefficients, are categorized as

water soluble and lipid or alcohol soluble. The results from this study indicate that the bioactive compounds in the test plant species are mostly lipid soluble.

Considering the powder formulations used in this study, insect mortality may be due to their physical action since the particles may block spiracles of the test insects and cause death by asphyxiation. Although there is no direct evidence of this in the present work, earlier works such as those of Ofuya and Dawodu (2002) showed that there is a direct relationship between particle size of plant powders and insect mortality in treated grains. Fine particle size aids even distribution of powders on the surface of seeds and the walls of the storage container thus increasing their possibility of making contact with the insects and killing them. In addition, plant powders cause abrasion of insect cuticle and lead to water loss (Sousa *et al.* 2005), which may cause stress and eventual death.

It appears that this study is the first report showing the oviposition suppressant and ovicidal effect of *C. ambrosioides* products and the lack of larvicidal activity against *C. maculatus*. Our work complements earlier reports on the insecticidal activity of *C. ambrosioides*, which are often centered on its toxicity against adult insects. This further highlights the potential of *C. ambrosioides* for practical control of insects by acting as oviposition suppressant and ovicide. The observed ovicidal action may be due either to the toxicity of volatile oil or physical action of non-volatile constituents. The volatile oil constituents may enter the egg through the funnel meant for gaseous exchange at the posterior pole (Credland 1992) and cause death of embryo. Alternatively the non-volatile constituents may block the funnel, prevent the exchange of gases and suffocate the embryo to death.

Finally, the present study showed that the bioactivity of *C. ambrosioides* against test insects was non-persistent. Such results indicate the need for repeated applications in order to provide effective protection of stored grains when *C. ambrosioides* products are used as protectants.

These results indicate that *C. ambrosioides* has potential for grain protection which can be harnessed as an alternative to synthetic insecticides.

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POLISH SUMMARY

TOKSYCZNOŚĆ PRODUKTÓW *CHENOPODIUM AMBROSIODES* L. (CHENOPODIACEAE) Z NIGERII PRZECIWKO TRZEM SZKODNIKOM PRZECHOWALNIANYM

W celu przebadania toksyczności ograniczenia składu jaj, działania jajobójczego i larwobójczego *Chenopodium ambrosioides* L. użytego w formie proszku, wyciągów i olejku eterycznego, przeprowadzono testy skierowane przeciwko *Callosobruchus maculatus* F. (Coleoptera, Bruchidae), *Sitophilus zeamais* Motschulsky (Coleoptera, Curculionidae) i *Tribolium castaneum* Jacqueline du Val. (Coleoptera, Tenebrionidae). Formulacja proszku była bardziej toksyczna dla *S. zeamais* niż dla *C. maculatus* lub *T. castaneum*, w czasie 48 godzin i wartości LC_{50} odpowiednio, w dawkach: 0,46 g/kg, 1,60 g/kg i 2,14 g/kg. Wyciąg etanolowy był bardziej toksyczny dla *C. maculatus*, w ciągu 48 godzin, przy LC_{50} 0,023 g/l niż dla innych gatunków owadów. Traktowanie olejkami eterycznymi wywołało wyższą aktywność fumigacyjną przeciw *C. maculatus* niż *S. zeamais* w przypadku wartości, odpowiednio, dla: 24 godzin i LC_{50} dla 1,33 μ l/l i 1,90 μ l/l. Para olejku wykazała aktywność przeciw jajom *C. maculatus*, ale nie miała możliwości do ustalenia śmiertelności. Utrata wagi ziaren zmieszanych z proszkiem z *C. ambrosioides* była niższa niż w kontroli po 150 dniach przetrzymywania w polu.