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Bioefficacy of Novaluron®, a chitin synthesis inhibitor against the tropical warehouse moth, *Ephestia cautella.*

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ABSTRACT:

The tropical warehouse moth, *Ephestia cautella* (Lepidoptera: Pyralidae) is a major pest of stored maize in Ghana. It is controlled mainly by the use of synthetic insecticides which has become a major challenge in the stored product industry in Ghana. Both laboratory and field trials were conducted to evaluate the efficacy of novaluron, a chitin synthesis inhibitor against *E. cautella*. Five concentrations of Novaluron (0.1, 0.2, 0.3, 0.4 and 0.5 mL/L of water) were prepared and each concentration was topically applied on the notal regions of 10 fifth instar larvae of *E. cautella* per concentration. At 0.4 mL/L and 0.5 mL/L treatments, larval mortality ranged between 50-80% after 96 h of exposure. Also, Novaluron (0.5 mL/L) was used to treat four surfaces (concrete, wood, glass and plastic) usually encountered in structural insect pest management systems and the larvae exposed to these surfaces. Hocklicombi® (5 mL/L) served as positive control. Larval mortality (35.5-97.5%), pupation (0.0-35.0%) and adult emergence (0.0-20.0%) in surfaces treated with Hocklicombi® compared favourably with those treated with Novaluron (25.0-97.5%), (2.5-60%) and (0.0-42.5%), respectively. A simulated field experiment was conducted in which four batches of 5 kg of maize in miniature bags were pretreated with 0.4 mL/L Novaluron and 50 unsexed adults were introduced. This was left in a crib at the University of Ghana farm for 60 days. The field experiment showed that after 60 days of storage there was a lower weight loss in the Hocklicombi® (6.6%) and Novaluron (6.8%) treatments compared to the negative control (11.3%).

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INTRODUCTION

Maize *(Zea mays)* is one of the major staple food crops in Ghana and it is susceptible to attack by several insect pests including the tropical warehouse moth, *Ephestia cautella* Walker (Lepidoptera: Pyralidae) (CAB1, 2006). *Ephestia cautella* larva feeds on stored products, damaging the product directly and form webs on the surface. The webbing contains larval excreta and exuviae which give unpleasant odour to the infested commodity. Older larvae may leave the food to find pupation sites in wall cracks.

In Ghana, *E. cautella* is controlled by the use of residual insecticides usually, synthetic pyrethroids and fumigants (CABI, 2006). The adverse effects of residual pesticides such as poisoning, environmental and health hazards and resistance development cannot be overemphasized (Obeng-Ofori, 2007). Hence the use of residual insecticides in stored product protection is challenging. There is therefore, the need for new cost and environment friendly alternatives with no adverse effect on non-target organisms (Obeng-Ofori, 2007; Arthur and Phillips, 2003). Some of these alternatives include botanicals, insect growth regulators, microbial pathogens among others (Arthur, 1996).

Novaluron (Rimon® 10 EC) is a benzoylphenyl urea group of insect growth regulators and a chitin synthesis inhibitor. Novaluron has been registered as an insecticide for food crops in several countries including South Africa, Australia and Ghana (WHO, 2003; EPA, 2006). In Ghana, novaluron has been successfully used in the laboratory against stored product pests such as the rice moth, *Corcyra cephalonica* Stainton (Lepidoptera: Pyralidae) (Sarbah, 2006), the red flour beetle, *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae) (Bakudie, 2006) and the tropical warehouse moth, *E. cautella* (Ibrahim, 2008).

In Ghana, most of the work done on novaluron focused on evaluating the effect of the chemical on different developmental stages of insects in the laboratory. There is no information on the use of novaluron for stored product protection in warehouses or cribs. *Ephestia cautella* is noted for feeding directly on the grains and also, the mature larvae leave the commodity in search of pupation sites in crevices, cracks and storage containers. Therefore, treating these surfaces to which the insect may be exposed will go a long way to mitigate the losses caused by this pest. Hence, screening Novaluron against *E. cautella* using the commodity and storage surfaces as substrates is crucial in the management of the pest. Such findings will contribute to the efforts by farmers and warehouse managers to reduce storage losses and contribute to the attainment of food security in Ghana.

This study presents laboratory and field tests that were carried out to determine the toxicity of novaluron to the 5th instar larvae of *E. cautella.* Other tests were also conducted to assess the efficacy of novaluron on different surfaces against immature stages of *E. cautella.*

MATERIALS AND METHODS

Insect cultures

The test insects were obtained from the Entomology laboratory of the Crop Science Department. Adult *E. cautella* were cultured on mixed substrate made up of wheat powder, maize flour and glycerol (5:5:1). Fifty adult *E. cautella* were introduced into each jar and left under laboratory conditions of 27±2°C and 55-60% relative humidity for 30 days to allow for the development of larval *E. cautella*. The set up was placed on trays containing industrial oil to prevent the crawling of other insects into the culture. The insects were reared and handled using ethically acceptable standard procedures in the laboratory.

Test chemicals

Novaluron (Rimon® 10EC), 1-[3-chloro-4-(1, 1, 2-trifluoro-2-trifluoromethoxy-ethoxy) phenyl]-3-(2, 6 difluorobenzoyl) urea, produced by Makhteshim-Agan Ltd (Israel) was used for the toxicity experiment and

Hocklicombi (Hockley International Ltd. Poynton, Stockport, U. K.) which contains 25% Fenitrothion and 5% Fenvalerate was used as a reference product.

Contact toxicity test

We adopted the method by (Eziah *et al.,* 2011). Concentrations of Novaluron (0.1, 0.2, 0.3, 0.4 and 0.5 mL/L) and 5 mL/L of Hocklicombi® were diluted in distilled water and used for the assays. Distilled water was used as negative control. Fifth instar larvae of both sexes were transferred into clean Petri dishes and the different dosages of the various concentrations was topically (1 µL) applied to the notal regions of the larvae using a micro applicator. Each experimental unit consisted of 10 larvae and was replicated for four times. The treated insect larvae were then transferred into glass petri dish containing food. The insect larvae were examined for mortality 24, 48, 72, 96 h, 7 days and 14 days after treatment. Criterion for death was as described by (Lloyd, 1969) in which insects were presumed dead when they failed to move in a coordinated manner after prodding with a blunt probe. Data collected include larval mortality, percent pupation and percent adult emergence were done after various treatments and exposure periods.

Surface treatment

The surfaces chosen for the study were concrete, plywood, glass and plastic which are among the common surfaces encountered in structural insect pest management. Individual concrete exposure arenas were created in square bottoms of plastic containers (6x6 cm) using a concrete patching material. Water-based slurry was prepared by mixing 1 kg of Portland cement to 2 kg of sand and 1 L of tap water and pouring 10 mL of the slurry into the bottom of the plastic container to create a treatment arena (Arthur, 1998b). Plywood arenas were made by cutting rectangular disks from 1.25 cm thick plywood to fit the plastic container then caulking the margins to prevent the larvae from escaping the surface. Plastic containers served as plastic surfaces and petri dishes were used as glass surfaces for the surface treatment.

Each of the four surfaces was treated with 4 mL of water (negative control treatment) or an aqueous solution of novaluron (0.5 mL/L) and Hocklicombi® (5 mL/L) (positive control). All treated arenas were allowed to dry overnight and fifth instar larvae (N=10) of *E. cautella* were exposed for 48 h. The larvae were then transferred to new petri dishes containing food under laboratory conditions of 27±2°C and 55-60% relative humidity. Post-treatment survival and mortality were recorded daily. Number of surviving larvae that successfully pupated and those that successfully emerged as adults were recorded.

Field experiment

Maize grains were obtained from the Madina (a suburb of Accra, Ghana) market and sieved to remove all debris. Maize grains (5 kg) were sterilized in the oven at 70°C for 3 h after which they were left in desiccators to cool. The grains were then treated with 0.4 mL/L Novaluron or 5 mL/L Hocklicombi®. These dosages had proven effective in laboratory experiments. Grains treated with distilled water served as negative control. Each treatment was replicated four times. Fifty unsexed adults of *E. cautella* were put onto the treated grains in each sack. The sacks were securely sealed by stitching and stored in a grain crib at the University farm for 60 days. Prior to their treatment, subsamples were taken from each sack for moisture and weight loss analyses using the standard volume method was carried out (Boxall, 1986). At the end of the storage period, the contents of the sacks were sieved. The number of both live and dead adult insects was recorded. Also, subsamples of the maize grains were collected for moisture and weight loss analyses as stated earlier.

Statistical analysis

Data involving percentages were arcsine transformed and were analyzed using the Analysis of Variance (ANOVA) with Genstat 9.2

(Lawes Agricultural Trust, 2007). Means were separated using the Least Significant Difference (LSD) test at 5% probability level.

RESULTS

Contact toxicity test

The percent larval *E. cautella* mortality following treatment with Novaluron and Hocklicombi® are presented in Table-1. Larval mortality varied with insecticide concentration and exposure period. Lower dosages of Novaluron (0.1-0.3 mL/L) caused less than 50% larval mortality after 96 h of exposure (Table 1). In contrast, novaluron concentrations of 0.4 mL/L and 0.5 mL/L caused between 50% to 80% larval mortality after 72 to 96 h of exposure. After 96 h exposure period, all dosages of Novaluron induced significantly (p = 0.05) higher larval mortality compared to the negative control. However, there was no significant difference in larval mortality between 5 mL/L Hocklicombi® and 0.5 mL/L Novaluron treatments. Also, novaluron applied at 0.4 ml/L and 0.5 mL/L did not differ significantly from each other after 96 h of exposure.

Pupation and adult emergence of *E. cautella* were observed in all insecticide treatments and the negative control with the exception of Hocklicombi® treatment. The percentage pupation in larvae treated with 0.1 mL/L Novaluron (57.5-65.0%) was not significantly different from the negative control (64.0-80.0%) (Figure 1). However, all other concentrations of Novaluron higher than 0.1 mL/L significantly (p = 0.05) impaired pupation. There was no significant difference in pupation 7 days after the exposure of *E. cautella* larvae to 0.5 mL/L Novaluron and 5 mL/L Hocklicombi®. Also, after 14 days, percentage pupation recorded in larvae treated with 0.4 mL/L and 0.5 ml/L was comparable.

All levels of Novaluron concentrations significantly reduced the development of F_1 of adult *E. cautella* (Figure 2). The highest adult emergence (77.5%) was recorded in the negative control and this differed significantly (p = 0.05) from all other novaluron concentrations applied. As concentration increased from 0.1 mL/L to 0.5 mL/L, adult emergence significantly (p = 0.05) reduced from 50 to 2.5%. Also, the effect of novaluron applied at 0.5 mL/L was comparable to Hocklicombi® treatment in impairing the development of adult *E. cautella.*

Surface treatment

Ephestia cautella larvae exposed on concrete surfaces treated with Novaluron showed a lower mortality than those exposed to concrete surfaces treated with Hocklicombi® (Table 2). Mortality was also lower on plastic and wood treated surfaces compared to Hocklicombi® treated surfaces. However, the percentage mortality of *E. cautella* on glass surfaces treated with

Table 1 Mortality of larval *E. cautella* (%) after treatment with novaluron and Hocklicombi® insecticides

Treatments (ml/L)	Mean±(s.e) % larval mortality (h)			
	24 h	48 h	72 h	96 h
Control (Water)	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
5.0 mL/L (HC)	87.5±0.1	87.5±0.1	87.5±0.1	87.5±0.1
Novaluron				
0.1	7.5±0.0	10.0±0.0	17.5±0.1	22.5±0.1
0.2	10.0±0.0	12.5±0.0	27.5±0.1	42.5±0.1
0.3	17.5±0.1	25.0±0.1	35.0±0.1	45.0±0.2
0.4	17.5±0.1	32.5±0.1	50.0±0.1	66.0±0.1
0.5	32.5±0.1	47.5±0.1	65.0±0.1	80.0±0.0
LSD (P < 0.05)	18.35	18.40	14.50	14.83

HC= Hocklicombi®
s.e = standard error

Table 2 Mortality of *E. cautella* larvae (%) after 7 days exposure on concrete, glass, plastic and wood surfaces treated with Hocklicombi® and novaluron insecticides

Mean (%) ± s.e mortality					
Insecticide	Type of surface				
	Concrete	Glass	Plastic	Wood	Means
Control	0.0 ± 0.0	0.0±0.0	2.5 ± 0.0	0.0 ± 0.0	0.7±0.0
Hocklicombi®	43.0 ± 0.1	97.5±0.0	45.0 ± 0.1	35.0 ± 0.1	55.0±0.0
Novaluron	25.0±0.1	97.5±0.0	17.5 ± 0.1	25.0 ± 0.1	41.1±0.0
Means	22.7± 0.0	64.7±0.0	21.7 ± 0.0	20.0 ± 0.0	-

LSD(P < 0.05): Main effects (insecticide = 1.21, surface= 1.39 Interaction (insecticide x surface)=2.4

novaluron was the same (97.5%) as those treated with Hocklicombi®. Surviving larvae were observed for pupation and adult emergence. Fewer *E. cautella* larvae pupated after exposure to concrete, plastic and wood surfaces treated with Hocklicombi® but no pupation was recorded on glass surfaces treated with Hocklicombi® (Table 3)

Fewer larvae pupated in glass surfaces-treated with novaluron and this was not significantly different from Hocklicombi®-treated glass surfaces. Generally, percentage adult *E. cautella* that emerged was greater on the untreated control for all the surfaces and differed significantly (p = 0.05) from all insecticide treated surfaces (Table 4). Mean percentage adult emergence of *E. cautela* observed on glass and plastic surfaces treated with novaluron and Hocklicombi® ranged from 0.0 to 25%. Thus, residual effects of novaluron and Hocklicombi® significantly reduced the development of *E. cautella* on glass and plastic surfaces.

Field experiment

Table 5 shows the dry weight loss of the treated grains after 60 days of storage using the standard volume method. Lower weight losses were observed in grains treated with insecticides (6.6-6.8%) compared to grains which were not treated (11.3%) using standard volume methods.

DISCUSSION

The present study showed that Novaluron concentrations of 0.4 mL/L and 0.5 mL/L significantly affected the metamorphosis of *E. cautella* to the adult stage. The effectiveness of novaluron at these dosages compared favourably with Hocklicombi®. The insect growth regulator's ability to regulate metamorphosis in the larvae through contact by topical application is consistent with its mode of action. Tomlin (2005) reported that novaluron was very effective on the larvae of insects when absorbed by ingestion and contact activity. The author also reported that the compound causes abnormal endocuticular deposition and abortive moulting.

Although pupation and adult emergence were observed in all treatment levels, most of the larvae treated with 0.4 mL/L and 0.5 mL/L Novaluron could not emerge into adults 23 days after treatment. This may be attributed to abnormal endocuticular deposition and abortive moulting in the larvae (Tomlin, 2005). Also, when cocoon covering the pupae were slightly removed, pupae found were malformed compared to those in the

Table 3: Percentage pupation of E. cautella after 14 days exposure on concrete, glass, plastic and wood surfaces treated with Hocklicombi® and novaluron insecticides

Means (%) ± s.e for pupation					
Insecticide	Type of surface				
	Concrete	Glass	Plastic	Wood	Means
Control	92.5±0.0	95.0±0.0	95.0±0.0	97.5±0.0	95.0±0.0
Hocklicombi	35.0±0.1	0.0±0.0	35.0±0.1	25.0±0.0	23.8±0.0
Novaluron	55.0±0.1	2.5±0.0	60.0±0.1	47.5±0.1	43.1±0.0
Means	61.9±0.0	41.2±0.0	63.1±0.0	56.2±0.0	-

LSD(P < 0.05): Main effects (insecticide5.6, surface= 6.6) Interaction (insecticide x surface)=13.20

control. Adults that emerged were found not to be active as those in the control. These findings are consistent with reports by Amos and Williams (1974). According to CABI (2006), pupal formation is completed in seven days and development from egg to adult ranges from 29-31 days under optimum conditions of 32.5°C and 70% relative humidity. However, in the present study under laboratory conditions of 27±2°C and 55-60% relative humidity, pupation extended up to 14 days and adult emergence was also delayed up to 30 days in the treated 5th instar larvae of *E. cautella*. Thus, novaluron was found to prolong the development period of *E. cautella* larvae to adults.

The ability of Novaluron to reduce the number of new generations is consistent with the findings of (Kostyukovsky *et al.,* 2003) and Kostyukovsky and Trostanetsky (2006). The authors found that novaluron applied at 1 ppm reduced the number of new generations of *S. oryzae* and *R. dominica* by 95% and also caused total mortality of the 3rd instar larvae of *T. castaneum*. The effectiveness of novaluron in preventing the metamorphosis of *E. cautella* when applied at 0.4 mL/L and 0.5 mL/L also confirms work done by Ibrahim (2008). The author found that development of *E. cautella* to adults was prevented when novaluron was applied at 0.4 mL/L and 0.6 mL/L.

These observations indicate that the effectiveness of novaluron as a grain protectant depends on the species of insect, dosage and exposure time. Wilson and Cryan (1997) and Mulla *et al*., (2003) stated that the effects of chitin synthesis inhibitors vary according to species, development stage, time of application, kind of compound and dose administered.

The residual effect of Hocklicombi® and Novaluron were significantly greater on glass surfaces than plastic, concrete or wood surfaces. Generally, Hockicombi® significantly caused higher mortalities on all the surfaces than novaluron. The high residual efficacy of Hocklicombi® may be attributed to the components of the compound. Hocklicombi® contains fenitrothion and fenvalerate as its active ingredients. These compounds have been reported by several researchers to have high residual effects when used as surface treatment against storage insects (Orui, 2004). Both compounds are non-systemic insecticides with contact and stomach activity (Tomlin, 2005).

In the present study, novaluron demonstrated excellent residual effect on glass surfaces by preventing the metamorphosis of *E. cautella* to the adult stage. The residual effect on glass surfaces treated with novaluron compared well with Hocklicombi®. However, on plastic, concrete and wood surfaces, Novaluron was less effective compared with Hocklicombi® but differed significantly from the untreated control surfaces. However, the residual effectiveness on plastic surfaces showed better efficacy than on concrete and wood surfaces.

The excellent effectiveness of Novaluron on glass and plastic surfaces is consistent with work done by (Atkinson *et al.,* 1992). The authors found that when hydropene, an insect growth regulator was sprayed on non-absorbent surfaces such as glass and ceramic tile, the

Table 4 Percentage adult emergence of *E. cautella* after 30 days exposure on concrete, glass, plastic and wood surfaces treated with Hocklicombi® and novaluron insecticides

Means (%) ± s.e for adult emergence					
Insecticide	**Type of surface**				
	Concrete	**Glass**	**Plastic**	**Wood**	**Means**
Control	92.5±0.0	90.0±0.0	95.0±0.0	97.5±0.0	93.8±0.0
Hocklicombi	20.0±0.1	0.0±0.0	12.5±0.1	12.5±0.0	11.2±0.0
Novaluron	42.5±0.1	0.0±0.0	25.0±0.1	42.5±0.1	27.5±0.0
Means	50.6±0.0	32.5±0.0	45.0±0.0	48.1±0.0	

LSD(P < 0.05): Main effects (insecticide5.9, surface= 6.34)= 6.34 Interaction Insecticide x surface=12.67

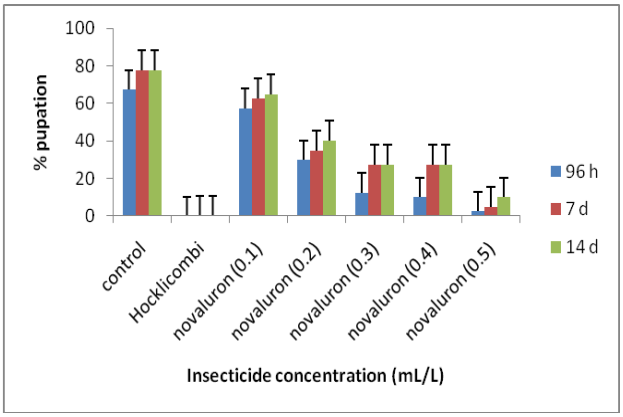

Figure 1 Percentage pupation (means±s.e) of *E. cautella* larvae after treatment with novaluron and Hocklicombi® insecticides. d = days h= hours

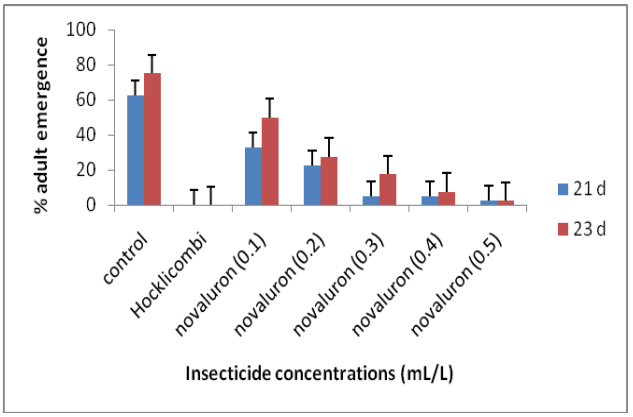

Figure 2 Percentage adult emergence (means±s.e) of *E. cautella* after treatment with novaluron and Hocklicombi® insecticides. d = days

survival, number of oothecae and percentage of cockroaches were more affected than on absorbent surfaces of finished plywood and fibreboard. The low mortality rates, pupation and adult *E. cautella* that emerged after exposure to concrete and wood surfaces in the current study can also be attributed to the composition of these surfaces. Burkholder and Dicke (1966) reported that new concrete surfaces contain high levels of alkaline which hydrolyze residues and reduce residual efficacy of insecticides hence, the low mortality rates on concrete-treated surface in the present study was not unexpected. Chadwick (1985) attributed low efficacy of insecticides on plywood surfaces to vaporization, chemical degradation, photodegradation and absorption of insecticides into surfaces. Thus, the low mortalities and higher survival rates observed in *E. cautella* exposed to wood surfaces treated with the insecticides may be due to the absorption of the insecticide into the wood surfaces after treatment.

In the field experiment, all the insecticide treatments significantly reduced dry weight loss in the grains compared to the control. Novaluron was observed to significantly reduce insect numbers in the treated grains and also had a significantly lower dry weight loss. Results from this study showed that novaluron effectively protected maize grains from damage by *E. cautella.* Grain weight losses calculated in the Novaluron treatment compared well with those observed in grains treated with Hocklicombi®. Considering that Novaluron selectively targets larval stages by inhibiting chitin synthesis and therefore, minimizes its impact on adults of non targeted insect species (Ishaaya *et al.,* 2001), Novaluron can be used in replacement of residual insecticides like Hocklicombi® for treatment of maize grains for storage.

Table 5 Percent dry weight loss after 60 days of storage using the standard volume method

Dosage (mL/L)	Mean dry weight loss (%)
Control	11.3±0.0
Hocklicombi 5	6.6±0.0
Novaluron 0.4	6.8±0.0

LSD(P < 0.05) = 1.63

CONCLUSION

The current study showed that Novaluron was effective in controlling the tropical warehouse moth. The application of Nuvaluron at 0.4 mL/L and 0.5 mL/L treatments resulted in larval mortality ranging between 50-80% after 96 h of exposure. Also, the treatment of concrete, wood, glass and plastic surfaces usually encountered in structural insect pest management systems with 0.5 mL/L Novaluron induced (25.0-97.5%) larval mortality, (2.5-60%) pupation and ((0.0-42.5%) adult emergence. These figures were comparable to those obtained from surfaces treated with 5 mL/L

Hocklicombi® insecticide. In the field maize treated with 0.4 mL/L Novaluron® and infested with adult *E. cautella* after 60 days of storage showed that there was a lower weight loss in the Hocklicombi® (6.6%) and novaluron (6.8%) treatments compared to the negative control (11.3%). This work has proven that Novaluron® could replace the synthetic insecticides that are used in the management of this pest and should be included in the management programmes for storage pests control.

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