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The use of essential oils for the control of *Callosobruchus subinnotatus* (Pic) in stored *Vigna subterranea* L.

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Abstract

Studies were conducted in the Crop Science laboratory, University of Calabar to evaluate the insecticidal actions of essential oils (EOs) of *Xylopia aethiopica, Dennetia tripetala, Pysostigma venenosum* and *Senna hirsuta* in the management of *Callosobruchus subinnotatus*. The EOs were extracted using soxhlet apparatus with n-Hexane as the solvent. Four concentrations (0.25%, 0.50%, 1.00% and 2.00 %) and n-Hexane as control were laid out in completely randomized design with three replications. Parameters assessed included repellency, fumigant action, weight loss as well as Lethal concentration (LC_{50}) of the treatments to the beetles at the lowest concentration of 0.25%. The EO of *Senna hirsuta* treated samples generally resulted in significantly ($P > 0.05$) lower weight loss than n-hexane treated samples. LC_{50} computation revealed that *D. tripetala* and *P. venenosum* (LC_{50} 0.22 at 48 hrs) were most efficacious against C. *subinnotatus*. The result supports the use of the test plants by small scale farmers in the protection of stored *V. substerranea* against *C. subinn*

Key words: Insecticidal action, repellency, contact toxicity, fumigant action, LC50, weight loss.

Introduction

There are various estimates of crop losses caused by storage insect pests which range from 10 - 40% in the hot, humid regions of the world (Phillips and Throne, 2010). Losses caused by insects include not only the direct consumption of kernels, but also accumulation of exuviate, webbing and cadavers. High levels of insect detritus may result in grains that are unfit for human consumption and loss of the food commodities, both in terms of quality and quantity (Meikle *et al*., 2002; Ukeh and Mordue, 2009). The major pests of stored grains and pulses of the sub-Saharan African region include those capable of penetrating and infesting intact seeds (grains) and have immature stages developing within the grains and secondary pests which feed on broken kernels, debris, and grains damaged by primary pests. Important among the primary pests are the pulse beetles, *Callosobruchus maculatus* and *C. subbinotatus* (Coleoptera: Bruchidae), the maize weevil, *Sitophilus zeamais* and rice weevil, *S. oryzae* etc. (Rees, 2004). The adults or larvae feed on the grains and eat the albumen or germ or both of them. The attack on the endosperm results in weight loss of the grains, reduction in nutrients and overall deterioration of their quality (Ukeh *et al*., 2010). Synthetic insecticides are often used in controlling insect pest in stored grains (Duke *et al*., 2003). The use of these pesticides is usually regarded as the panacea to pest problems in stored grains in order to feed the alarming human population growth. However, these insecticides pose health hazards to mammals and the environment. Their use is further limited by the lack of technical know-how of farmers, traders and consumers in handling these poisonous insecticides. There is thus, the need to search for alternative methods of pest control in all stages of agricultural production including storage. There is need for the application of less hazardous and safe alternatives that are locally and readily available in nature, cheap and affordable to farmers, simple and convenient to use, specific to the target species and are generally environmentally friendly (Isman, 2006; Umoetok *et al*., 2009). The objectives of the study were to investigate the mode of action (eg. Repellent, contact toxicity and fumigant action) of Guinea Pepper (*Xylopia aethiopica*), Pepper Fruit (*Dennettia tripetala*), Stinking Cassia (*Senna hirsuta*), Calabar Ordeal Bean or "Eseri" Bean (*Physostigma venenosum*) against *Callosobruchus subinnotatus* in *V. substerranea* L.

Materials and methods

Description of the study location

The research was conducted under laboratory conditions in the Department of Crop Science, University of Calabar, Cross River State, Nigeria. The study area is located at geographical coordinate of Latitude 4^0 57^1N of Equator and Longitude $8^0$$19^1$E of Greenwich Meridian, with an altitude of 37m a.s.l (Iloeje, 2001). Calabar is characterized by two distinct moist tropical climates of wet (April-November) and dry (December-March) seasons, respectively. It has an annual rainfall of between 2200 – 3700 mm, average relative humidity of 89%, an annual air temperature range of 26–30 ^{0}C

and lies along the humid coastal region of South Southern Nigeria (Iloeje, 2001).

Collection of plant materials and extraction of essential oils

The plant materials used for the trial were; Guinea Pepper (*Xylopia aethiopica*), Pepper fruit (*Dennettia tripetala*), Calabar bean (*Physostigma venenosum*) and Stinking Cassia (*Senna hirsuta*). The fruits of *Xylopia aethiopica D. tripetala* were obtained from Akpabuyo Local Government Area of Cross River State, while seeds of *S. hirsuta* and *Physostigma venenosum* were collected from fallow lands in Calabar Municipal area in Cross River State. The plant materials were cleaned, air dried under shade for 3 days and preserved in the freezer until they were needed for the various experiments. Fifty grams (50 g) of the dried portion of each plant material was ground and oil extracted with n-Hexane (50nml).

Fifty millimeters (50 ml) of N-Hexane was measured into 500 ml round bottom flask containing the plant powder and extracted using Soxhlet. The plant extract was put into a beaker and evaporated in water bath at 80 ^{0}C. The process was repeated until the required volume of oil needed was obtained.

Insect Culturing of insects

Callosobruchus subinnotatus was cultured on 500 g dry untreated Bambara groundnut seeds which were obtained from local farmers in Yala Local Government Area of Cross River State. The adults of *C. subbinotatus* were obtained from the laboratory stock culture maintained in the Department of Crop Science, University of Calabar, Calabar. About 100 g of Bambara ground seeds were put into kilner jars. Twenty unsexed adult *C. subinnotatus* were introduced into each jar. The covers of the jars were replaced with wire mesh to facilitate air circulation. The insect culture was maintained at room temperature in the laboratory. The adults were allowed to oviposit in the containers for 3 days, after which they were removed. The culture was left for 35 days and the new beetles emerging from each culture jars were sieved out for the experiment.

Repellence bioassays

The repellency test was adopted from the method of McDonald *et al.* (1970) as modified by Talukder and House (1995) and reported by Liu *et al.* (1999). Each plant essential oil was tested for its repellence activity against *C. subinnotatus*. Four concentrations of the four plant essential oils (0.25, 0.50, 1.00 and 2.00%) were obtained and prepared for use in the experiment by diluting each essential oil at 0.05, 0.10, 0.20 and 0.40 ml in 20 ml of n-hexane, respectively. Whatman No. 1 Filter paper (9cm diameter) was cut into two equal parts and placed in the Petri dishes (diameter 8cm) at 2 cm apart. Half part was treated with the plant essential oils and the remaining half treated with the solvent (n-Hexane). Ten (10) unsexed adults each of *C. subinnotatus* were introduced at the center of the Petri dishes, in between the filter papers and the Petri dishes were arranged on the laboratory bench in a Completely Randomised Design (CRD), replicated three times, under a relatively dark environment to minimize the effect of light and the high activity of *C subinnotatus*. The number of beetles on both sides of the filter papers were recorded from each petri dish after 30mins, 1 and 2 hrs treatment application. Based on the number of insects which stay on the treated and untreated sides of the filter, repellency was determined. Percentage repellency was calculated by the equation:

$$\text{Repellency (\%)} = \frac{(C - T)}{(C)} \times 100$$

Where C = No. of insects collected from the untreated filter papers

T = No. of insects collected from the treated filter papers

Fumigant toxicity bioassay

The various concentrations of the plant essential oils were obtained as described earlier. Strips of filter papers were soaked in the different concentrations of the four plant essential oils (i.e. 0.25, 0.50, 1.00 and 2.00% V/V) in beakers. The treated filter paper strips were allowed to dry for 3 minutes and then placed against the wall of a 100 ml flat bottom glass flask. Ten *C. subinnotatus* adults were introduced separately into the treated paper strips in the flask and the bottle sealed with screw caps. Filter papers soaked with n-hexane only served as control. The 18 treatments were laid out in a completely randomized design (CRD) with 3 replications. Mortality was determined at 1.5 hrs. and 3 hrs after treatment. Adults were considered dead if appendages did not move when probed with a Carmel hair brush. Percentage mortality was calculated and probit analysis used to estimate the lethal concentration (LC_{50}) values.

Weight loss bioasay

Hundred grams (100 g) each of maize seeds and Bambara groundnut were weighed out into separate transparent plastic containers. The seeds in each container were treated with 20 ml of each essential oil at different concentrations (0.25, 0.50, 1.00 and 2.00% V/V). The seeds and essential oil were thoroughly mixed. Twenty unsexed 3-day old *C. subinnotatus* were introduced into the admixture of oil and the seeds. Similarly, containers with seeds mixed with 20 ml n-hexane only served as controls. Thus, in each experiment, there were eighteen treatments replicated 3 times to give 54 experimental units laid out in a Completely Randomized Design (CRD). The containers were covered with nylon mesh and their perforated lids screwed in place to ensure confinement of the insects. Data on weight loss were taken cumulatively for 12 weeks. On each occasion, the insects and the produce were separated from the powder emanating from each container due to insect activities, the seeds weighed with a top load electronic weighing balance. Percentage weight loss was obtained by using the formula:

$$\frac{(W_I - W_S)}{(WI)} X\ 100$$

Where W_I = Initial weight of grain before storage
W_S = Weight of grain after storage at a specified time.

Results

Repellency of the four essential oils to *C. subinnotatus*

The repellent effects of the different plant essential oils (Eos) evaluated at different concentrations and exposure time on *C. subinnotatus* are presented in Table 1. Exposure of the beetles for 30 minutes to the different concentrations of oils resulted in an increase in repellency which was dose-dependent. It was observed that, an increase in the concentration of *P. venenosum* and *S. hirsuta* from 0.25% to 0.50% significantly ($p< 0.05$) resulted in higher repellence to the bean beetles. However, when the concentration was increased from 1.00% to 2.00%, *S. hirsuta* caused higher repellence than the other oils. A hundred percent (100%) repellence was obtained with *P. venenosum* at 2.00% concentration. The repellence of the beetles for an exposure period of 1 hour followed a similar trend as when it was exposed for 30 minutes. There were no significant ($p>0.05$) differences in repellency among the plant EOs at the lowest concentration which resulted in increase in percentage repellence with the exception of *P. venenosum*. Increase in EO concentration from 0.25% to 1.00% significantly increased percentage repellence of the bean weevil. *Xylopia aethiopica* EO at 0.50% was as effective in repelling *C. subinnotatus* as other plant essential oils at 1.00%. There were no significant ($p>0.05$) differences in repellence among the different oils when applied at the highest concentration of 2%. No significant ($p>0.05$) difference was also observed when the test insect was exposed to all the essential oils at 2 hours. Percentage mortality of adult *C. subinnotatus* exposed to varying concentrations of different plant essential oils applied as fumigant. The results of the fumigant toxicity of the plant essential oils (EOs) on the mortality of

bean weevil (*C. subinnotatus*) at different exposure period are presented in Table 2. When the bean weevil was exposed to the EOs for 1.5hrs, there was no significant ($p>0.05$) difference in weevil mortality between *X. aethiopica, D. tripetala* and *S. hirsuta* at both 0.25% and 0.50% concentrations respectively. The fumigant test showed that higher concentrations (1.00 and 2.00%) of the plant oils resulted in most cases to no significant ($p>0.05$) percentage mortality of the weevil than at lower levels of 0.25 and 0.50% respectively. No significant ($p>0.05$) mortality was observed when *X. aethiopica* and *D. tripetala* were applied at 0.25% and 0.50% and *D. tripetala* at 0.50% and *S. hirsuta* at 0.25%. Generally, the fumigant toxicity effect increased with increase in the period of exposure of the weevil to the different concentrations of the plant EOs. However, total mortality of the bean weevil was not achieved in any of the plant EOs within the 3.00hrs exposure period. (Table 2).

Variation in LC_{50} values of essential oils by contact toxicity

The LC_{50} values of the different plant essential oils against adults *C. subinnotatus* at different period of exposure by contact toxicity are shown in table 3. The LC_{50} values consistently decreased with increase in the time of exposure to *P. venenosum*. However, for the other plant materials the trend was not consistent from 3 to 6 hrs exposure period but from 12 to 48hrs, there was consistent decrease in the LC_{50} values. At 3 hrs of exposure, the least LC_{50} values was recorded when the bean beetles were tested against *X. aethiopica,* however, at 6 hrs the essential oil of *S. hirsuta* had the least LC_{50} value. At 24 and 48 hrs, *P. venenosum* EO had the least LC_{50} value. Generally, at 48hrs of exposure, the LC_{50} value for all the plant EOs was low with *D. tripetala* and *P. venenosum* having the lowest.

Effect of plant essential oils on weight loss of treated Bambara groundnut by *C. subinnotatus*.

The results on the evaluation of the efficacy of plant essential oils in reducing weight loss in Bambara groundnut is presented in Fig 1. The same trend were observed at 12 weeks after application (WAA) when higher percent weight loss was recorded on the control as against samples treated with essential oils. Generally, there was a reduction in percent weight loss with increase in both concentrations of the EOs.

Table 1: Repellent effects (%) of different plant essential oils (EOs) against *C. maculatus* at different time of exposure

			Time of exposure (Hours)	
Plant essential oils	Conc. (%) 0.5		1.00	2.00
X. aethiopica	0.25	33.33e	22.22e	11.11ed
D. tripetala		49.20e	22.22e	65.74abc
P. venenosum		33.33e	33.33de	73.67bc
S. hirsute		41.27e	22.22e	41.27bcde
X. aethiopica	0.5	73.67bcd	69.05abc	30.16cde
D. tripetala		69.05cd	61.11bc	84.25a
P. venenosum		49.20e	33.33de	49.20abcde
S. hirsute		49.20e	49.20cd	57.14abc
X. aethiopica	1.0	79.63abcd	75.00ab	49.20abcde
D. tripetala		84.25abcd	69.05abc	77.38ab
P. venenosum		88.88abc	69.05abc	55.16abc
S. hirsute		60,05d	69.05abc	55.16abc
X. aethiopica	2.0	96.29ab	92.59a	61.11abc
D. tripetala		96.29ab	87.96a	69.44abc
P. venenosum		100.00a	88.88a	59.79abc
S. hirsute		92.59abc	92.59a	69.05abc

Means within a column followed by the same letters are not significantly different according to Duncan's

Table 2: Percent mortality of adult *C. subinnotatus* exposed to varying concentration of different plant essential oils applied as fumigant

		Time of exposure (Hours)			
Plant essential oils	Conc. (%)	1.5		3.00	
X. aethiopica	0.25	3.33	(6.13 f)	3.33	(6.13 g)
D. tripetala		10.0	(15.00 ef)	16.67	(19.93 fg)
P. venenosum		33.33	(34.93 cde)	56.67	(48.93 cde)
S. hirsuta		13.33	(21.13 def)	20.00	(26.07 efg)
X. aethiopica	0.5	3.33	(6.13 f)	6.67	(12.27 fg)
D. tripetala		63.33	(53.07 abc	86.67	(72.80 abc)
P. venenosum		40.00	(38.87 bcd)	60.00	(51.13 bcde)
S. hirsuta		60.00	(50.87 abc)	36.67	(37.13 def)
X. aethiopica	1.0	40.00	(39.77 bcd)	63.33	(53.33 bcde)
D. tripetala		76.67	(61.73 ab)	93.33	(81.13 ab)
P. venenosum		70.00	(57.00 abc)	86.67	(68.87 abc)
S. hirsute		83.33	(66.13 a)	83.33	(70.07 abc)
X. aethiopica	2.0	66.67	(54.80 abc)	66.67	(60.00 abcd)
D. tripetala		96.67	(60.00 abc)	96.67	(83.87 a)
P. venenosum		90.00	(51.00 abc)	100.00	(90.00 a)
S. hirsute		90.00	(71.60 a)	10.00	(90.00 a)
Control		0.00	(0.00 f)	0.00	(0.00 g)
Hexane		0.00	(0.00 f)	0.00	(0.00 g)

Means within a column followed by the same letters are not significantly different according to Duncan's

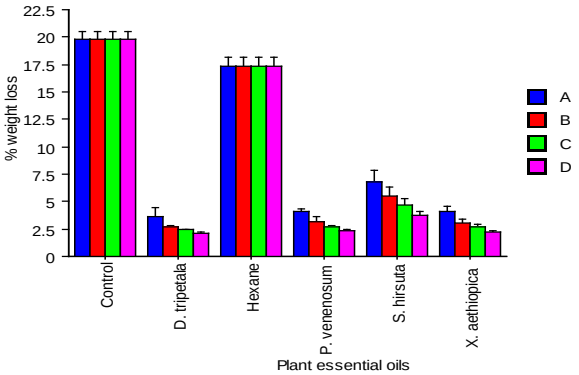

Fig.1: Effect of different plant essential oils on percentage (%) weight loss of Bambara groundnut infested with *C. subinnotatus* at 12 Weeks after application.
Key: A = 0.25%, B = 0.50%, C = 1.00%, and D = 2.00%

Table 3: Percentage variation in LC_{50} values with respect to the duration of exposure of *C. subinnotatus* to the essential oils of different plants during contact toxicity test

	Duration of exposure (hrs)				
Plant materials	3	6	12	24	48
X. aethiopica	1.84	11.76	2.65	1.04	0.50
D. tripetala	2.59	19.32	5.32	0.78	0.22
P. venenosum	18.87	4.32	2.58	0.36	0.22
S. hirsute	2.53	5.17	2.29	1.69	0.37

Discussion

The results obtained from this study showed that the fruits of *X. aethiopica and D. tripetala* and the seeds of *P. venenosum* and *S. hirsuta* had varying levels of insecticidal action against *C. subinnotatus*.

When compared with the control, all the plant essential oils were effective in reducing the population and activities of *C. subinnotatus* under laboratory condition. Their effectiveness was dependent on cconcentration and exposure period except in repellence bioassay where short exposure period resulted in higher repellency than prolonged exposure. This explains why mortality was at highest concentration of 2.00% at 2hrs, for repellency bioassay and 12 wks for weight loss evaluation respectively. Dose related mortalities in similar treatments have been reported by earlier authors (Kieta *et al.,* 2000; Law-Ogbomo, 2007; Ukeh *et al*., 2012). Ogunwenmo *et al*. (2007) reported that plants have phytochemicals that act as chemical defense against other organisms thus, the strong odour produced by the oils of these plants and their chemical composition may have been responsible for the mortalities observed on the insects (Lee *et al.,* 2007; Kouninki *et al*., 2007). The bioactivity of these plant essential oils were due to fumigant action and contact toxicity of the oils to the insects. At higher concentrations, the oils may have blocked the insect spiracles thus, disrupting respiration and resulting in suffocation (asphyxiation) and death as reported earlier by Oparaeke and Kuhiep (2006). The results of the present study confirm reports by Lajide *et al*. (1995), Ejechi and Akpomedaye (2005), Adedire and Akinkurolere (2005), Rayapakse (2006), Kouninki *et al*. (2007) and Asawalem *et al*. (2012) that *Dennitia tripetala*, *Xylopia aethiopica* and other tropical plants species have strong anti-feedant and anti-survival effects on different storage pest including weevils and beetles. Phytochemical screening and isolation of *X. aethiopica* according to Lopez-Martin *et al*. (2002) revealed the presence of alpha-pinene, beta-pinene, 3-carene and terpinene-4-ol, while that of *D. tripetala* revealed the presence of beta-phenylnitroethane, alkaloids, dennettine, three phenanthrine alkaloids (identified as uvariopsine), stephenanthrine, argentinine, phenolics and vanillin. The presence of these anti-oxidant and semio-chemicals must have been responsible for the acute toxicity of the essential oils (EOs) to the bean beetles. These findings on the effect of contact toxicity on *C. subinnotatus* are consistent with other reports on essential oils that exhibited insecticidal activity on stored product pests (Hall and Harman, 1991; Adedire *et al*., 2011). The results of percentage mortality of adult *C. subinnotatus* exposed to varying concentrations of plant EOs applied as fumigant revealed that, all the plant essential oils resulted in significantly ($P<0.05$) higher percentage mortality than the control treatment with hexane. The EOs of *D. tripetala* and *S. hirsuta* at 0.50% concentration were as effective as the highest concentration (1.00 and 2.00%) of *X. aethiopica*. These agree with other researchers on the use of plant essential oils as fumigants in the control of stored product pests (Kieta *et al.,* 2000; Law-Ogbomo, 2007; Adedire *et al*., 2011; Ukeh *et al*., 2012). The results of this study showed a very good potential for the use of the four plant essential oils as fumigants. Repellents in the form of essential oils, powders or distillates have the potential for the exclusion of stored-product pests from grains and they have been used to prevent insects from feeding and oviposition (Asawalem *et al*. 2012; Ukeh *et al.,* 2012). The presence of certain chemical compounds in the essential oils which altered the behaviour of *C. subinnotatus* as a result of the effect of the oils on the olfactory sensilla of the insect's antennae, Several workers including Javid and Poswal (1995), Talukdar and Howse (1995), Tapondjou *et al*., (2002) and Ukeh *et al.,*(2009) had reported that n-hexane or ethanol extract of *D. tripetala* could individually result in 40.1–100% repellence when used in protecting stored dry fish or grains from beetles. All the plant essential oils tested in this study were highly repellent to the bean beetles. Omar *et al.* (2007) reported feeding deterrence of *Cosmopolites sordidus* due to *D. tripetala* extract while anti-feedant effect was reported by Lajide *et al.* (1995). On weight loss, results obtained from the experiment showed that there was a significant ($P<0.05$) increase in weight loss in the untreated (control) and hexane treated samples irrespective of exposure time.. Kieta *et al*. (2003), Tripathi *et al*. (2002) and Singh and Yadav (2003) reported that various oils used as seed treatments against storage pests are effective in reducing damage (weight loss). The different effects between the plant oils used in the present study may be due to the type of plants and the composition of their different active ingredients, but all the tested plant oils were effective in reducing weight loss of stored Bambara groundnut.

The results of the LC_{50} values of different plant essential oils indicated that with prolonged exposure time, the potency of the EOs are increased. Also, it is apparent that the efficacy of the essential oils

varied among the plant materials. The active ingredients in these plant materials and the physiological mechanism of interference in the insects may possibly have accounted for this variations. From the LC_{50} values obtained, fumigation of the grains with the plant essential oils should be considered compared with spraying or just rubbing with the oils. It is possible that the volatile active components of the oils could easily permeate and penetrate the grains vis-a-avis the bean beetle, than when it is rubbed or sprayed. It was observed that the efficacy of the botanicals were dose-dependent with higher doses resulting in higher mortalities of the *C. subinnotatus*. Graphs of percentage mortality versus log of concentrations were constructed and the LC_{50} was computed for each essential oil. Results of the LC_{50} revealed that *D. tripetala* and *P. venenosum* (LC_{50} 0.22 at 48hrs) were the most efficacious against *C. subinnotatus*. Photochemical screening from literature revealed the presence of several active compounds in the essential oils of these plants and may probably be responsible for the bio-insecticidal properties of these oils and the observed mortalities.

Conclusion

Results obtained from this research revealed that the essential oils *X. aethiopica, D. tripetala, P. venenosum* and *S. hirsuta* were toxic and effective in controlling Bambara groundnut beetle, *C. subinnotatus*. They could therefore be incorporated into the integrated pest management practices by the local farmers to reduce damage caused by insect pests.

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Influence of Abiotic Factors on the Efficacy of Insect Growth Regulators Against *Trogoderma Granarium* (Everts)(Coleoptera: Dermestidae)

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ABSTRACT

Present study was designed to investigate the effects of different combinations of three temperatures (20, 25 and 30°C) and three relative humidity levels (55, 65 and 75%) on the efficacy of three synthetic IGRs i.e., pyriproxyfen, lufenuron and buprofezin at concentrations of 1, 5 and 10ppm on fecundity and adult emergence inhibition of *T. granarium* under controlled laboratory conditions. This study was conducted at Grain Research Training and Storage management Cell, Department of Entomology, University of Agriculture, Faisalabad, Pakistan. All the treatments were replicated three times using Completely Randomized Design. Larvae of *T. granarium*were exposed to IGRs at different levels of temperature and relative humidity. F_1 adult emergence results showed that at temperature 20°C, the highest percent reduction in adult emergence (84.38, 70.65 and 79.94%) was recorded after exposure to lufenuron, buprofezin and pyriproxyfen treated diet, respectively. At 75% relative humidity, lufenuron, buprofezin and pyriproxyfen caused 77.53, 80.00 and 80.32% reduction in adult emergence, respectively. Adults were exposed to IGRs at different temperature and relative humidity to evaluate the oviposition inhibition. The results revealed that at temperature 20°C, maximum percent reduction in fecundity (87.95, 80.45 and 70.55%) was recorded after exposure to buprofezin, pyriproxyfen and lufenuron treated diet, respectively. At 75% relative humidity buprofezin, pyriproxyfen and lufenuron caused 86.73, 83.72 and 69.11% reduction in fecundity, respectively. It is concluded that temperature and relative humidity play an important role in the effectiveness of insect growth regulators.

Key words: Temperature, Relative Humidity, *Trogoderma granarium,* Insect Growth Regulators, Efficacy