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Efficacy of Insect Growth Regulators against Red Tef Worm, *Mentaxya ignicollis* (Walker) (Lepdoptera: Noctuidae)

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Abstract

Tef (*Eragrostis tef* (Zucc.), Trotter: Poaceae) is a staple food crop of Ethiopia where it is originated and diversified. Red tef worm (*Mentaxya ignicollis*) is a serious pest of tef grown on clay soils. Hence the present study emphasized on the evaluation of insect growth regulators to control Red Tef Worm. Laboratory study were carried out in completely randomized design with two insect growth regulators (lufenuron at doses 20, 40 and 60g a.i/ha and teflubenzuron at doses of 75, 112.5 and 150g a.i./ha) against 3rd instar larvae of RTW. The green house study was carried out in randomized complete block design with the two Chitin synthesize Inhibitors (CSIs) (lufenuron at dose of 40g a.i./ha and teflubenzuron at dose of 112.5g a.i./ha). Efficacy of CSIs in affecting the hatchablity of the eggs was also studied. From the laboratory and greenhouse experiments the IGRs, lufenuron and teflubenzuron, caused mortality after affecting the developmental stage of RTW larvae and also inhibited egg hatchability. In general, lefenuron (40g a.i./ha) was found to be effective and and showed high potency against 3rd instar larvae of RTW under laboratory and greenhouse conditions. Since the CSIs are safe to the environment and other beneficial organisms, it is recommended to be verified for usage under open and large field conditions for the control of RTW.

Key words*:* Lufenuron, Red tef worm , Teflubenzuron

Introduction

Tef (*Eragrostis tef (*Zucc.*),* Trotter: Poaceae) is a staple food crop of Ethiopia, where it is originated and diversified. Over 2.8 million hectares of land is covered with tef every year with a predicted 1228 $kgha^{-1}$ mean productivity at national level (CSA, 2011).

Red tef worm (RTW) (*Mentaxya. ignicollis)* is a serious pest of tef grown on black or heavy, deeply cracking clay soils. The status of red tef worm, *M. ignicollis* as a major pest of tef was reported from Shewa, Kefa, Gojam, in some places in Tigray and Wollega regional states of the country (Tadesse, 1987). It can cause up to 30% loss in yield (IAR, 1986).

Control measures of RTW, including cultural, chemical and microbial methods have been attempted to some extent (Tadesse, 1987a, 1987b). However, they were not adequate to minimize the density of RTW and thereby alleviate the yield loss caused by the pest. On the other hand, use of synthetic insecticides causes environmental pollution, pest resistance and toxicity to other non-target organisms.

Previously, no research has been done with insect growth regulators to control RTW. Chitin synthesis inhibitors, lufenuron and teflubenzuron, are extensively available nowadays and are being tested both in the laboratory and field condition (Arnold *et al*., 2009; Kai *et al*., 2009; Tassou and Schulz, 2009; Mansur *et al*., 2010).

Hence in this research, insect growth regulators, lufenuron and teflubenzuron were used in both laboratory and greenhouse studies on RTW to provide information, assist the development of an integrated pest management program and provide management options for the farmer.

Therefore, the present study was carried out under laboratory and greenhouse conditions to measure efficacy of insect growth regulators (lufenuron and teflubenzuron) and determine effective dose against the larvae of *M. ignicollis* under laboratory and greenhouse conditions.

Materials and Methods

Growing of tef plants on pots

The tef variety (Kuncho) was sown on pots at the recommended rate of 25$kgha^{-1}$.The sizes of the pots were 18x30cm. The pots were filled with clay, compost and sandy soil in the ratio of 1:2:1 respectively. The experiment was carried out at Ambo Plant Protection Research Center (APPRC).

Rearing of red tef worm (RTW)

The larvae of red tef worm were collected from infested tef fields in South West Shoa Zone, Becho and

Saden Sodo woredas early in the morning on tef plants at grain filling stage. The collected larvae were transferred to plastic bowls which were quarter filled with mixture of fine sand and black soil and were provided with fresh tef seedlings every 24 hours and kept under temperature of 26±2^{o}C.

The larvae pupated in the plastic bowls at the depth of 3-9cm. The soil in the plastic bowls with pupae were wetted and kept undisturbed. On an average, 15 days after pupation adults started to emerge. To culture the adults, tef seedlings were grown on small pots and kept in the cage (1.5m x1.5m). Then emerged adults were carefully transferred to the cage with 3:1, female to male ratio and provided with 10% sugar solution (Tadesse and Matthews, 1986) by sprinkling on the tef seedlings, placing cotton wool soaked in sugar solution in small cups in the cage as well as suspending cotton wool which was wetted with the solution. Every day, the sugar solution was sprinkled and the cotton in the cups was changed. As an alternative zigzag shaped paper were suspended on the corner to facilitate oviposition. Three days after emergence, adults started oviposition. The eggs were laid on the underside of tef leaves, on the suspended paper, and on the surface of cage (nylon cage). Ten to fourteen days after oviposition, eggs hatched and the larvae fed on the leaves of the seedlings.

Efficacy of Lufenuron and Teflubenzuron in the laboratory

Two insect growth regulators (lufenuron 50% EC and teflubenzuron 15% SC) and endosulfan were obtained from Abel Agrisher Ethiopia PLC. And were evaluated at three rates each by using sterile distilled water: teflubenzuron 15% SC (150, 112.5 and 75 g a.i.ha^{-1}) and lufenuron 50% EC (60, 40 and 20 g a.i.ha^{-1}). Endosulfan 35%EC at 700g a.i. / ha and unteareted checks were used. The doses were chosen from a preliminary trial carried out on related and other insect species (N.S.Butter *et al*., 2003 and Bakr et*. al*., 2008).

Bio-assay on larvae

Total of 240 3rd instar larvae were used for this experiment. Fresh chopped tef leaves were kept in each Petri dish (12.5cm diameter). Ten third instars larvae were transferred to each Petridish and the treatments were sprayed using hand sprayer on the leaves and on the surface of the larvae. Larvae were allowed to feed the treated leaves for 24 hours (Bakr, *et.al*, 2008). The control insects were allowed to feed on untreated leaves. All the treatments were kept under the same laboratory condition. The experiment was carried out in a completely randomized design with eight treatments in three replications. Fresh chopped leaves of tef were replaced every day. Larval mortality was recorded every 24 hours for ten consecutive days.

Bio-assay on egg

Three hundred twenty eggs were used for the experiment and were obtained from laboratory reared *M. ignicollis.* Doses of 112.5 g a.i.ha^{-1} and 40 g a.i.ha^{-1} were prepared for teflubenzuron and lufenuron, respectively. These doses were selected from the the preceding laboratory based on their effectiveness against the larvae. Sixteen Petri dishes of 12.5cm diameter lined with filter paper were prepared. Twenty black headed eggs which develop to larvae were transferred into each Petri dish carefully using camel brush.

Individual treatments were applied topically to eggs. Control eggs were treated with sterilized distilled water and the standard check, endosulfan 35%EC was applied at 700 g a.i ha^{-1}. The treated eggs were kept at the temperature of 27$\pm$1^{o}C, 65-85% RH and 12L: 12D photoperiods until larval hatch. Hatchability percentage was recorded every 24 hours for five consecutive days after application. The embryocidal effect of the treatments on developing embryo was calculated as the percentage of embryos that died in the eggs.

Verification of IGRs in greenhouse

The experiment was conducted at APPRC, entomology greenhouse in a randomized completely block design with three replications.

The treatments were: Teflubenzuron@ 112.5g a.i/ha, Lufenuron@40g a.i/ha, Endosulfan@ 700g a.i/ha and Untreated check.

Ten third instar larvae of RTW were placed on each tef plants pot at grain filling stage. The treatments were applied using hand sprayer, early in the morning. Larval mortality was assessed every 24 hrs for 10 consecutive days after treatment application.

Data analysis

Larvae and egg mortality under each treatment in both laboratory and greenhouse conditions was corrected using Abbott (1925) formula and the corrected mortality data of the IGRs in laboratory and greenhouse conditions were analyzed using one way analysis SAS program (SAS, 2005). LD_{50} and LT_{50} were also calculated using SAS probit analysis.

CM (%) = (T-C)/(100-C)*100
Where, CM is Corrected mortality
T is Percent mortality in treated larvae of RTW
C is Percent mortality in untreated larvae of RTW

Results and Discussion

Efficacy of Lufenuron and Teflu-benzuron against 3rd Instar Larvae of RTW under Laboratory and Greenhouse Conditions.

The results of laboratory study showed that there were significant differences in larval mortality between untreated check and the other treatments (Table 1). Lufenuron at the dose of 40 and 60 g a.i./ha caused significant mortality of 96.29 and

100%, respectively when compared with the other treatments; however, no significant differences was observed with standard check (endosulfan(100%)). On the other hand, there was no significant differences between teflubenzuron at 112.5g a.i./ha and 150g a.i/ha and the lowest dose of lufenuron, 20g a.i/ha (84..26%). This indicated that lufenuron was more effective than teflubenzuron in causing mortality of RTW.

The data on lufenuron and teflubenzuron potency against the 3rd instar larvae of RTW in greenhouse are presented in Table 2. Both IGRs caused significant mortality of the larvae ten days after treatment application, compared to the untreated control. Lufenuron (94.45%) was not significantly different from the standard check (97%), but teflubenzuron (80.56%) was inferior. Significant differences ($p<0.001$) between lufenuron (94.45%) and teflubenzuron (80.56%) with respect to the larval mortality were also observed from the results.

Most of the dead larvae treated with lufenuron and teflubenzuron were dark and shriveled and the old exoskeletons were still attached to the lower part of the abdomen. Prior to death, the treated larvae remained motionless and were unable to feed on the provided tef seedlings. Ratnakaran *et al*., (1985) justified that the inability of larvae treated with chitin synthesis inhibitors insect growth regulators to feed on the leaves could have been caused by the displacement of the mandible and labrum or the blockage of the gut. Fogal(1977) also reported that the incomplete clearance of the larval gut at moult as well as the reduced amount of chitin in the newly moulted mouth parts could prevent the larvae of *Diprion similis* from feeding after ecdysis. The symptoms exhibited by the treated RTW larvae were consistent with symptoms reported for some other species of insects such as *Lucilia cuprina, Manduca sexta* and *Lymantria dispar* treated with chitin synthesis inhibitors (Abdel-Monem *et al.* 1980; Kotze, 1992; Root and Dauterman 1996)

Nagesh and Varma (1997), who reported that the application of lufenuron on diamond back moth caused high percentage of mortality in larvae compared with teflubenzuron. Kim *et al*., (2000) have shown also that lufenuron was highly effective (>80% efficacy) against diamondback moth larvae. Ivan *et al*., (2011) reported that lufenuron showed high toxicity against larvae of *S. littoralis* in comparison with tebufenozide. Lufenuron caused 100% mortality in larvae that were fed with food containing a high concentration of the compound (0.01 ppm) (Ivan *et al*., 2011). Within a 24 hour period from the beginning of precocious molting, the larvae developed elongated heads, and stopped feeding. In this “sleeping stage” the larvae died after 2–3 days (Ivan *et al*., 2011). Lufenuron exhibited more efficiency on both 2nd and 4th larval instars of *H. armigera* in laboratory bio-assays in terms of toxicity and speed of kill compared with flufenoxuron and triflumuron (Arnold *et al*., 2009). This study also agreed with that of Abdel Rahman *et al*., (2007) when they tested the direct and latent effects of lufenuron and a lufenuron mixture on the development of S. littoralis larvae and reported that lufenuron has toxic effects on tested larval instars.

Based on this study, the comparative effects of lufenuron and teflubenzuron on the 3rd larval instar of RTW indicated that lufenuron has the potential to kill the larvae more effectively than teflubenzuron.

Table 1. Cumulative Percent Mortality of 3rd Instar Larvae of RTW when Treated with Lufenuron and Teflubenzuron under Laboratory Condition

Treatments	Means(+SE)
Teflubenzuron @ 75g a.i.ha^{-1}	76.75+0.93d
Teflubenzuron @ 112.5 g a.i.ha^{-1}	81.02+3.24cd
Teflubenzuron @ 150 g a. i.ha^{-1}	88.43+0.46bc
Lufenuron @ 20 g a. i. ha^{-1}	84.26+4.63cd
Lufenuron @ 40 g a.i.ha^{-1}	96.27+3.7ab
Lufenuron @ 60 g a. i.ha^{-1}	100+0.00a
Endosulfan@700g a.i.ha^{-1}(standard check)	100+0.00a
Untreated check	13.33+3.33e

CV=5.82%

Means followed by the same letters are not significantly different by Student Newman Keuls (SNK) test (P<0.001)

Table 2. Percent Mortality of 3rd Instar Larvae of RTW Treated with Lufenuron and Teflubenzuron in Greenhouse

Treatments	Means(+SE)
Teflubenzuron (112.5g a.i./ha)	36.25+1.25b
Lufenuron (40g a.i./ha)	91.25+1.25a
Endosulfan(700g a.i./ha)	92.5+1.45a
Untreated control	17.5+1.45c

CV=7.87% Means followed by the same letters are not significantly different by Student Newman Keuls (SNK) test (P<0.001)

Lethal dose determination

The results showed that lufenuron was more effective than teflubenzuron, as it had lower LD_{50} (9.88) and LD_{90} (24.79) values (Table 3). The relative potency values indicated that lufenuron was more effective than teflubenzuron with 1.21 and 12.15 times great potency against 3rd instar larvae of RTW at the LD_{50} and LD_{90} level respectively than teflubenzuron.

El-sayed et al. 2011, reported that the relative potency values indicated that lufenuron was more effective than flufenoxuron and triflumuron with 2.5 and 9.5 times great potency at the LD_{50} level, respectively, and 3 and 5.8 times higher potency than flufenoxuron and triflumuron at the LD_{90} level, respectively.

In agreement with this study, the comparative effects of lufenuron, flufenoxuron and triflumuron on the 2nd and 4th larval instar of *Spodoptera littoralis* indicate that lufenuron has the potential to kill *Spodoptera littoralis* larvae more efficiently than flufenoxuron and triflumuron. And it is also likely to be more efficient in the field compared with the other tested insecticides (El-Sayed *et al*.2011). The efficiency of lufenuron, teflubenzuron and flufenoxuron against third and fifth instars of *Spodoptera littoralis*, were also investigated by Bayoumi *et* *al*. (1998) under laboratory conditions. They showed that third instars are more sensitive to lufenuron.

The present study indicated that the lufenuron was more toxic than teflubenzuron to 3rd instar of RTW larvae. Therefore, it is recommended to use the lower dose of lufenuron than teflubenzuron to bring more larval mortality of RTW.

Table 3. LD_{50} and LD_{90} of Teflubenzuron and Lufenuron against Larvae of RTW

Treatments	LD_{50} (95% CI) [a]	LD_{90}(95% CI)	Relative potency [b]	
			LD_{50}	LD_{90}
Teflubenzuron	11.95	301.19	1.21	12.15
Lufenuron	9.88(4.08-13.84)	24.79(20.14-30.09)	1	1

[a] LD_{50} or LD_{90} and 95% fiducial limits (CLs) are given in g of a.i.

[b] Relative potency is calculated as LD_{50} or LD_{90} of the tested IGRs/LD_{50} or LD_{90} of the most effective IGR

Lethal time determination

The median lethal time (LT_{50}) values of lufenuron, teflubenzuron and endosulfan tested on the 3rd larval instar of RTW are shown in Table 4. The time required for 50% mortality decreased with increasing dose in both tested CSIs, however, there is no dramatic changes from lufenuron 40 to 60g a.i./ha on 3rd larval instar which were 3.55 and 3.38 days respectively. Similarly, at high doses of lufenuron (60g a.i/ha) and teflubenzuron (150g a.i/ha) approximate days (3.38 and 3.82 respectively) to kill 50% of the larvae was observed; however, at their lower doses, 20 and 75g a.i./ha respectively, lufenuron caused 50% larval death within 4.91 days where as teflubenzuron caused within 5.37 days, which means that lufenuron is more toxic when both are used at their lower dose. Endosulfan caused 50% death within not more than one day. This indicated that it is more toxic to 3rd instar larvae of RTW.

The result indicated that lufenuron exhibited more efficiency in killing 50% of 3rd instar larvae of RTW faster than teflubenzuron at lower dose, but slower than endosulfan at any doses. At their individual high doses, they showed almost similar toxicity to the larvae within the days not more than four. On the other hand the result of medial lethal time indicated that lufenuron is more toxic to 3rd instar larvae of RTW, since it caused 50% mortality at the dose less than half (60g a.i./ha) of teflubenzuron (150g a.i./ha) in the same days interval. This data show that there is no need for using very high concentrations of lufenuron to get the pest controlled. Generally, lufenuron is preferred than teflubenzuron economically, because it cause immediate 50% mortality at lower dose.

Table 4. LT_{50} and LT_{90} of Teflubenzuron and Lufenuron to 3rd Instar Larvae of RTW under Laboratory Condition

Treatments	LT_{50} (95%CL)[a]
Teflubenzuron@75g a.i.ha^{-1}	5.37(4.99 - 5.76)
Teflubenzuron@112.5g a.i.ha^{-1}	4.35 (3.95- 4.76)
Teflubenzuron@150g a.i.ha^{-1}	3.82 (3.43- 4.20)
Lufenuron @ 20 g a. i. ha^{-1}	4.91 (4.38 - 5.48)
Lufenuron @ 40 g a.i.ha^{-1}	3.55 (3.20 - 3.89)
Lufenuron @ 60 g a. i.ha^{-1}	3.38 (3.07 - 3.68)
Endosulfan@700g a.i.ha^{-1}	0.983374

[a] LT_{50} and 95% fiducial limits (CLs) are given in days

Potency of Teflubenzuron and Lufenuron against egg hatchablity of RTW

Effect of teflubenzuron and lufenuron was examined (Table 5). Eggs of RTW were observed unhatched when treated with the CSIs and the standard check. Shrinkage, death of 1st instar larvae in the egg and partial hatch (part of larvae were attached with the body of the egg) were the symptoms observed during the experiment (Fig.1). On the contrary, normal 1st instars larvae were hatched in the untreated eggs. Sallam (1999) reported that the developed embryos were unabled to perforate the surrounding vitelline membrane, it could be due to a weakened chitinous mouth parts that was insufficiently rigid to effect hatching. Ovicidal activity of the tested CSIs in the present study could be due to the disturbance in cuticle formation of the embryo. Ivan *et al*., (2011) also reported that reduced hatchability resulted from numerous changes occurring in the course of embryonic development.

Table 5. Percent Unhatched Eggs of RTW when Treated with Lufenuron and Teflubenzuron under Laboratory Condition

Treatments	Means(±SE)
Teflubenzuron (112.5g a.i./ha)	36.25±1.25b
Lufenuron (40g a.i./ha)	91.25±1.25a
Endosulfan(700g a.i./ha)	92.5±1.45a
Untreated control	17.5±1.45c

CV=4.55%

Means followed by the same letters are not significantly different by Student Newman Keuls (SNK) test (P<0.001)

From ANOA results, significant differences (P<0.001) between treatments in affecting the egg hatchability were observed. However all treatments were significantly different from the untreated control, there was no significant differences between lufenuron (91.25%) and endosulfan (92.5%) in inhibiting the hatchability of the egg of RTW. On the other hand, lufenuron is highly significant difference from teflubenzuron (36.25%) to affect egg hatchability.

Reports from previous studies found that the exposure of diamondback moth eggs to different concentrations of teflubenzuron led to significant inhibition of egg hatching when compared with other IGRs (Karimzadeh *et al.*,2007 ; Hayens and Smith, 1993;Perng *et al*., 1988) in contrary, the present study indicated that teflubenzuron was inferior to lufenuron and endosulfan to inhibit egg hatchability. Osman and Mahmoud (2008) observed that 88.3% reductions of cotton leafworm eggs 24 h after treatment with lufenuron when compared with control. Sammour *et al.* (2008) also reported 73.2% reduction in egg hatchability of the same insect. Therefore, the results justified that eggs of RTW were highly affected by lufenuron and endosulfan; however, teflubenzuron is alternatively preferable than untreated control to inhibit the egg hatchability of RTW.

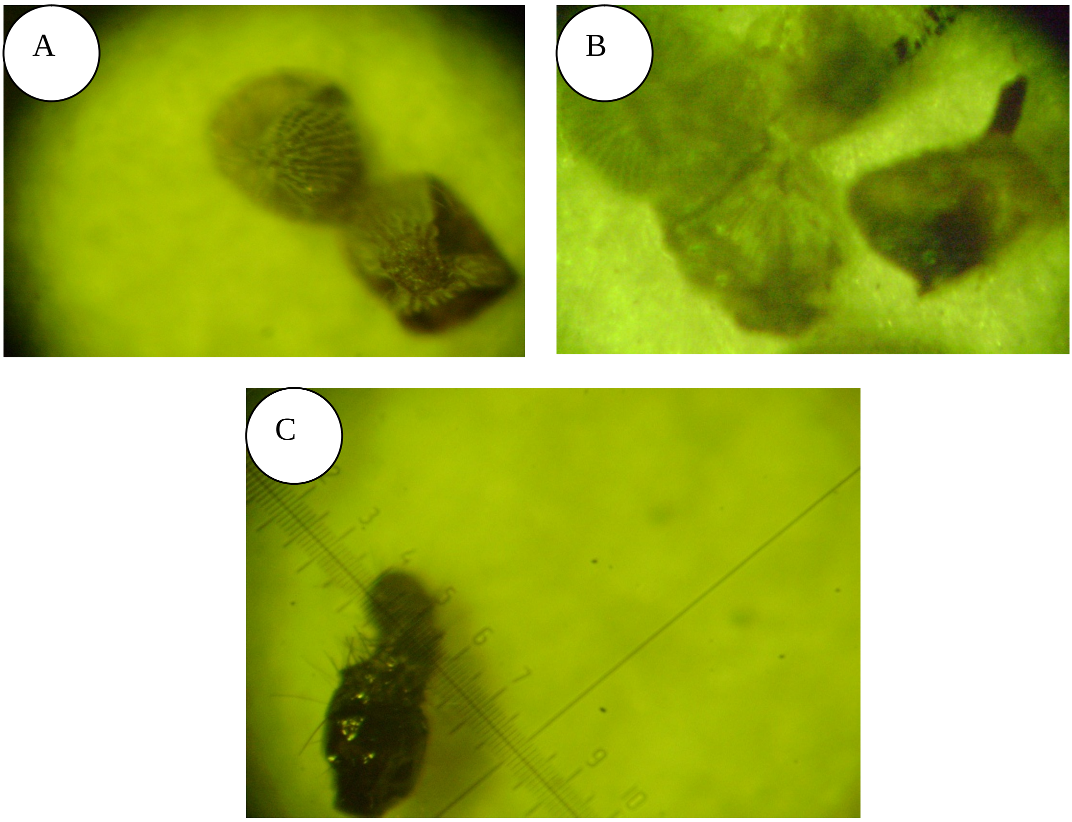

Figure1. A, B and C are the Effect of Lufenuron, Teflubenzuron and Endosulfa, Respectively on Egg Hatchability of RTW

Conclusions

The comparative effectiveness of lufenuron and teflubenzuron on 3rd larval instar of RTW showed that lufenuron was more effective than teflubenzuron, as it has lower LD_{50} (9.88) and LD_{90} (24.79) values. The relative potency values indicated that lufenuron was more effective than teflubenzuron with 1.21 and 12.15 times great potency against 3rd instar larvae of RTW at the LD_{50} and LD_{90} level, respectively than teflubenzuron. Lufenuron caused highly significant egg hatchability inhibition of RTW. Generally, the total efficiency for laboratory and green house experiments indicated that lufenuron and teflubenzuron caused mortality of RTW larvae and inhibited egg hatchability. However, lufenuron caused high mortality at lower dose. It can therefore be concluded that, because of its safety to environment and other beneficial organisms, lufenuron can be used at dose of 40g a.i./ha for further study under open and large field conditions for the control of RTW.

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