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Side Effects of Fungicides on the Egyptian Cotton Leafworm Infesting Potato Plants

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ABSTRACT

The side effects of seven fungicides used on potato plants to control early blight disease on Egyptian cotton leafworm (ECLW) were tested. By leaf dipping method, the fungicides were applied on castor bean leaves at the same rate of the recommended rate by MoA for controlling early blight disease. Feeding response, survival, larval and pupal growth and development to the adult stage were calculated under laboratory conditions. Also, the effect of these fungicides on the first generation was also recorded. The results indicated that all these treatments significantly reduced the feeding of 4th instar larvae when fed for 24h on treated leaves with the tested fungicides. Also, reduced larval weight and increased larval duration (days). Also, reduced pupation % and pupal weight (mg) and increased pupal duration. These fungicides reduced the number of egg/female. These treatments reduced the longevity of male and female and finally these fungicides affected on first generation of this insect. These ICLW exhibited antifeeding properties and affect the growth and development of Egyptian cotton leaf worm (ECLW).

Keywords: ECLW-fungicides-side effects. Biological aspects - toxicity - Potato.

INTRODUCTION

The Egyptian cotton leafworm [WCLS], *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae), is one of the most important polyphagous pests, widely distributed in the Mediterranean region, North and East Africa, Asia and Europe (Quero ., 2002). Also, this insect is considered one of the most destructive agricultural lipidopterous pests of field and many vegetable and ornamental plants in Egypt and other countries in the world (Hosny ., 1986; Saleh ., 2015 and Wahba, 2016), causing considerable damage by feeding on leaves, fruiting points, flower buds and, occasionally on the bolls (Metay, ., 2015 and Abdien, Salma ., 2016).

Previous reports indicated that the fungicides may be causes different effects on insects (Marchas S. and Lauge, 1997; Adamski and Ziemnichi, 2004; El-Kholy, 2005; Pratissoli ., 2010; Delpuech, J.M. and R. Alemand (2011) and El-Sisi ., 2016 and Srivastava, K ., 2017).

The present study was carried out to determine how food consumption, growth and development of the ECLW, *S. liltornlis* may be influenced by feeding on leaves treated with the chemical fungicides used on potato plants to control early blight disease.

MATERIALS AND METHODS

The laboratory susceptible strain (LSS) of *Spodoptera littoralis* were obtained from the Plant Protection Research Institute (PPRI), Agricultural Research Center (ARC), Dokki, Giza, Larvae of the ECLW were reared on clean and fresh cast or bean leaves , *Ricinus communis* L, in the laboratory of the department of 25±2^{o}C and 65±5% R.H. with a photoperiod of 12:12h (L:D) according to El-Defrawi ., (1964).

Seven commercial fungicides were used at the recommended field rate to control early blight disease on potato plants. Some characteristics of these potato fungicides are listed in Table (1).

Table 1. Trade common names and rates of application of the used fungicides.

Trade names	Concentrations and formulation	common names	Rate of application 100L. water
Anadol	80% WP	Mancozeb	250 gm
Duett- M	73% WG	Mancozeb +cymoxanil	125 gm
Index	73% WP	Copper hydroxide	250 gm
Optima	25% EC	Difenoconazole	50 cm^{3}
RemiK	30% WG	Dimethomorph+ Metalaxyl	200 gm
Roxyl plus	68.9% WP	Copper hydroxide + Metalaxyl	150 gm
Sandcure	72% WP	Mancozeb + Metalaxyl	250 gm

According to the recommendations by Agricultural pesticide committee (APC), Ministry of Agriculture and Land Reclamation, (2017).

By leaf dipping bioassay method, the clean and fresh castor bean leaves (*R.communis* L.) were dipped in each fungicides for 15 sec., and placed on paper towel to dry at room temperature. Each fungicide had 10 replicates and each replicated included 10 starved larvae. Other replicates were dipped in water only and served as control treatment.

Neonated 4th instar larvae (one day old) with average 20±2 mg were placed in each replicate. The larvae were fed on treated leaves for 24h, after which they fed on untreated leaves until pupation, fresh leaves were kept in a similar rearing Jar under the same conditions to estimate the natural loss of moisture, which was used for calculating the corrected weight of consumed leaves. The feces and dead larvae were discarded. At the end of larval instar, dead larvae were discarded. The uneaten leaves and larval mortality was observed daily, uneaten leaves and new fresh leaves were weighted daily after taking the natural loss of moisture in consideration (control 2).

At the end of larval stage, the consumed fresh leaves was corrected according to a method described by Ghanema, Hoda (2002) as follow:

Corrected weight of the consumed leaves= Cb / Ca x Ta.

Where:

Cb= Initial weight of castor bean leaves before larval exposure.

Ca= Final weight (after exposure to natural dryness for 24 hrs.) of leaves without larvae

Ta= Final weight of treated leaves after feeding the larvae for 24 hrs.

Daily weight (fresh basis) of consumed treated leaves/larva=A–B/C.

Where:

A= Initial fresh weight of treated leaves before feeding the larvae.
B= Corrected fresh weight of treated leaves after feeding the larvae.
C= Number of survived larvae.

Also, the following parameters were calculated at the end of larval instar as follow:

Mean weight of consumed leaves larva^{-1} (gm).

- **Feeding ratio (Wada and Manukata, 1968) = b / a x 100.**

Where:

a= Amount of fresh weight of leaves consumed in the control.
b= Amount of fresh weight of leaves consumed in the treatment

- Antifeedant index % (AFI) according to Pavela . (2008) as follow:

AFI={(C-T)/(C+T)} x 100.

Where:

C= Weight of leaves consumed in the control.
T= Weight of leaves consumed in the treatment.

- The larval weight (mg).
- The larval duration (days).
- The larval mortality %.

Also, at pupal stage, the pupation %, pupal mortality% and pupal duration (days) were recorded. At the adult stage, the adult emergence % was calculated.

Pairs of 2 males and 2 females resulted from each treatment of any fungicides were placed in glass jar (2 liter) containing *Nerium oleander* L., leaves as a site of egg laying. The jars were provided with pads of cotton soaked with a 15 % sugar solution and covered with muslin. Pads of cotton were replaced daily when needed. Five replicates were used in each treatment beside the control. The effect of these fungicides on adults was recorded as follow:

- Reproductive parameters (fecundity and fertility).

Where:

Fecundity= Number of deposited eggs female^{-1} .
Fertility= Egg hatchability %.

- Sterility % (Toppozada ., 1966) as follow:

= 100 - {(a x b / A x B) x 100}.

Where:

a= Number of eggs laid female^{-1} in the treatment.
b= Percent of hatchability in the treatment.
A= Number of eggs laid female-1 in the control.
B= Percent of hatchability in the control.

Percentages of mortality were corrected when needed according to Abbott's formula (Abbott, 1925). The latent effects on the first generation were also recorded as mortality %.

Statistical analysis:

Statistical analysis was conducted by ANOVA and compared by L.S.D. test at 5% and 1% of probability in all experiments (Duncan, 1955).

RESULTS AND DISCUSSION

The data represented in Table (2) indicated that the effect of seven commercial fungicides on feeding response of fourth instar larvae of the Egyptian cotton leaf worm (FCLW), *Spodoptera littoralis* (Boisduval) [Lepidoptera: Noctuide]. These results showed that the effect of the tested fungicides on weight of consumed leaves larva^{-1} (gm), feeding ratio % and antifeed ant index % (AFI). The 4th instar larvae were feed on treated leaves by the recommended field rate for 24hrs. then, on untreated leaves of castor bean until the end of larval instar or pupation. The data revealed that all the tested fungicides significantly (p=0.5) reduced the weight consumed leaves larva^{-1} (gm) in comparison with the untreated control. In this respect, sand cure fungicide significantly was the most effective in reducing the consumed leaves larva^{-1} at the end of larval instar, followed by Roxy plus fungicide, Remik, optima, Anadol, Index and Duett-M, respectively. The feeding ratio% for these fungicides was 91.21, 89.74, 87.55, 86.81, 86.81, 84.61 and 83.15% for Duett-M. Inedex, Anadol, Remik, Optima, Roxy plus and Sandcure, respectively. Also, antifeedant index % was recorded and the efficacy of the tested fungicides was recorded follow: sand cure (9.20%), Roxy plus (08.33), optima and Remik (07.06% for each), Inadol (06.64%), Index (05.41%), and Duett-M (04.60%).

Table 2. Food consumption by *Spodoptera littoralis* larvae after feeding of 4th instar for 24hrs. on fungicides treated leaves (at the end of larval instar).

Treatments	Rate 100L. Water	Average weight of consumed leaves larva^{-1} (gm) ± SE*	Feeding ratio %	AFI(%)**
Anadol 80% WP	250 gm	02.39 ± 0.055 b c c	87.55	06.64
Duet-M73% WG	150 gm	02.49 ± 0.049 b	91.21	04.60
Index 77% WP	250 gm	02.45 ± 0.026 c b	89.74	05.41
Optima 25% EC	50 cm^3	02.37 ± 0.080 b c c	86.81	07.06
Remik 30% WG	200 gm	02.37 ± 0.051 b c c	86.81	07.06
Roxyl puls 68.9% WP	150 gm	02.31 ± 0.042 c d	84.61	08.33
Sandcure 72% WP	250 gm	02.27 ± 0.038 d	83.15	09.20
Untreated	-	02.73 ± 0.062 a	100.00	00.00

Means with the same letter are not significantly different. * SE = Standard error. ** AFI = Antifeeding index.
L.S.D. for Treatments 5% = 0.15 & 1% = 0.20

The obtained results in Table (3) cleared that the effect of the tested fungicides on larval weight (mg), larval duration (days) and larval mortality % at the end the larval instar. The data clearly indicated that all the tested fungicides significantly (p=0.050 reduced the larval weight (mg) at the end of larval instar compared with the untreated control. Also, the results clearly indicated that No significant differences was observed (p=0.05) between the tested fungicides. The effect of the tested fungicides on larval durations (days) at the end of larval instar was also recorded. These results showed that sand cure fungicide significantly (p=0.05) was the most effective in increasing the larval duration (12.81), followed by Roxy plus (12.36), Anadol (12.33), Index (12.32), optima (12.28), Duett-M (12.01). No significant differences between Remik (11.89) and untreated treatment (11.71), but the other fungicides were significant (p=0.05) than untreated treatment. The larval mortality % were 22.0, 21.0, 18.0, 15.0, 14.0, and 13.0% for sand cure, Roxy plus, optima, Remik, Index, Duett-M and Anadol (13.0%).

The data presented in Table (4) revealed that the effect of seven fungicides on pupal duration (day) and pupal mortality%. The data indicated that all the tested fungicides significantly (p=0.05) reduced pupal weight (mg) in comparison with the untreated control. The sandcure fungicide was significantly (p=0.05) was the most effective in reducing the pupal weight which gave (375.240, followed by Roxy plus (387.78), Anadol (388.76), Renik (390.77), Index (391.85), optima (404.11) and Duett-M (709.84), respectively, the effect of the tested fungicides on pupal duration (days) was recorded. The data clearly indicated that sand cure and Anadol fungicide significantly (p=0.05) increased pupal duration (days) in comparison with other treatments. All fungicides increased pupal duration relatively and these was more obvious in Anadol and Sand cure respectively (Table 4). The pupal mortality % was also recorded.

Table 3. Effect of feeding of *Spodoptera littoralis* 4th instar larvae for 24 hrs. on fungicides treated leaves on larval instar (at the end of larval instar)

Treatments	Rate 100L. Water	Average larval duration (mg) ± SE*	Average larval duration (days) ± SE	Larval mortality %
Anadol 80% WP	250 gm	1454.6±15.28 b	12.33±0.081b	13.00
Duet-M73% WP	150 gm	1471.6±10.86 b	12.01±0.054 b c	14.00
Index 77% WP	250 gm	1470.8±13.35 b	12.32±0.082 b	15.00
Optima 25% WP	50 cm³	1440.0±6.07 b	12.28±0.065 b c	18.00
Remik 30% WP	200 gm	1468.4±08.18 b	11.89±0.053 d e	17.00
Roxyl puls 68.9% WP	150 gm	1480.0±36.16 b	12.36±0.070 b	21.00
Sandcure 72% WP	250 gm	1463.0±09.30 b	12.81±0.076 a	22.00
Untreated	-	1547.0±18.62 a	11.71±0.200 e	00.00

Means with the same letter are not significantly different. * SE = Standard error. ** AFI = Antifeeding index.
L.S.D. for Treatments at 5% = 48.87 and 0.28 ; at 1% = 65.70 and 0.37

Table 4. Effect of feeding of *Spodoptera littoralis* 4th in star larvae for 24 hrs. on fungicides treated leaves on pupal stage.

Treatments	Rate 100L. Water	Pupation %	Average larval duration (mg) ± SE*	Average larval duration (days) ± SE	Larval mortality %
Anadol 80% WP	250 gm	87.00	388.76±03.59 c	11.61±0.26 a	04.60
Duet-M73% WP	150 gm	86.00	409.84±02.86 b	10.15±0.16 c b	05.81
Index 77% WP	250 gm	85.00	391.85±02.72 c	10.54±0.31 b c	07.06
Optima 25% WP	50 cm³	82.00	404.11±02.86 b	10.39±0.14 b c	09.76
Remik 30% WP	200 gm	83.00	390.77±05.53 c	10.57±0.29 b c	08.43
Roxyl puls 68.9% WP	150 gm	79.00	387.78±02.53 c	10.70±0.40 b	11.39
Sandcure 72% WP	250 gm	78.00	375.24±02.61d	11.60±0.14 a	11.54
Untreated	-	100.00	425.32±05.59 a	09.97±0.07 c	00.00

Means with the same letter are not significantly different. * SE = Standard error.
L.S.D. for Treatments at 5% = 10.79 and 0.71; at 1% = 14.51and 0.95

The effect of the tested fungicides on reproductive parameters (Fecundity and fertility) of adult stage was illustrated in Table (5). The effect of these fungicides on fecundity was observed and these results indicated that sand cure fungicide was the most effective in reducing the eggs female^{-1} followed by Roxy plus and Remik. All fungicides significantly (p=0.05) reduced the fecundity in comparison with the untreated control. The fertility (hatchability%) was also affected by these compounds. The hatchability % was reduced by sand cure (85.830, Optima (87.25), Index (88.71), Roxy plus (90.26), Duett. M (90.33), Remik (19.12) and Anadol (91.91), respectively. the same trend was also observed in the case of sterility.

Table 5. Effect of feeding of *Spodoptera littoralis* 4th instar larvae for 24 hrs. on fungicides treated leaves on adult stage.

Treatments	Rate 100L. Water	Adult emergence%	Average larval duration (mg) ± SE*	Average larval duration (days) ± SE	Larval mortality %
Anadol 80% WP	250 gm	94.40	806.0±05.46 c b	91.19	14.03
Duet-M73% WP	150 gm	94.19	829.32±05.39 a b c	90.33	12.37
Index 77% WP	250 gm	92.94	833.28±18.96 a b c	88.71	12.57
Optima 25% WP	50 cm³	90.24	845.74±12.77 a b	87.25	13.68
Remik 30% WP	200 gm	91.57	821.16±11.15 b c	91.12	12.47
Roxyl puls 68.9% WP	150 gm	88.61	815.96±13.97 b c	90.26	13.85
Sandcure 72% WP	250 gm	88.46	795.00±14.24 a	85.83	20.18
Untreated	-	100.00	866.40±28.25 a	98.67	00.00

Means with the same letter are not significantly different. * SE = Standard error.
L.S.D. for Treatments at 5% = 43.15; at 1% = 58.02

The data presented in Table (6) showed that the effect of seven fungicides on longevity of adults (males and females). The results clearly indicated that all the tested fungicides reduced the male and female longevity (days). In male longevity, the results indicated that all the tested compounds significantly (p=0.05) reduced the male longevity (days) compared with the untreated control. Sand cure fungicide was the most effective followed by Roxy plus, Anadol, Remik, Index, Duett-M and Optima, respectively. The reduction % for the above mentioned compounds was 15.31, 11.22, 10.07, 9.25, 5.16, 4.67 and 3.03%, respectively. The results also indicated that all the tested fungicides reduced the female longevity. The reduction % was 13.37, 11.56, 10.18, 07.76, 05.18, 04.05

and 02.85 for Roxy sand cure, Remik, Anadol, Index, Duett-M and Pitma, respectively.

The results in Table (7) showed that the effect of the tested fungicides generation of the *S.littoralis* insect. These data indicated that the latent effect of fungicides was observed clearly on larval, pupal and adult stages. These fungicides were affected on first generation, and the most effective fungicided was sand cure (33.77), followed by Roxy plus (31.36), Optima (24.21), Remik (21.12), Index (20.07), Anadol (17.73) and Duett. M (16.09), respectively.

Table 6. Effect of feeding of *Spodoptera littoralis* 4th instar larvae for 24 hrs. on fungicides treated leaves on longevity of adults.

Treatments	Rate 100L. Water	Adult emergence%	Average larval duration (mg) ± SE*	Average larval duration (days) ± SE	Larval mortality %
Anadol 80% WP	250 gm	10.98±0.21 c	10.07	10.69±0.23 b c d	07.76
Duet-M73% WP	150 gm	11.64±0.16 b	04.67	11.12±0.07 a b	04.05
Index 77% WP	250 gm	11.58±0.15 b	05.16	10.99±0.12 b c	05.18
Optima 25% WP	50 cm^3	11.84±0.13 a b	03.03	11.26±0.28 a b	02.85
Remik 30% WP	200 gm	11.08±0.18 c	09.25	10.41±0.20 c d e	10.18
Roxyl puls 68.9% WP	150 gm	10.84±0.09 c	11.22	10.04±0.30 e	13.37
Sandcure 72% WP	250 gm	10.34±0.019 d	15.31	10.25±0.14 e d	11.56
Untreated	-	12.21±0.16 a	00.00	11.59±0.23 a	00.00

Means with the same letter are not significantly different. * SE = Standard error.
L.S.D. for Treatments at 5% = 0.47 and 0.61; at 1% = 2.74 and 0.81

Table 7. Mortality percentages of the developmental stages of the first generation previously exposed the 4th instar larvae of *Spodoptera littoralis* to fungicides treated leaves.

Treatments	Rate 100L. Water	Larval mortality %	Pupal mortality %	Adult mortality %	Accumulative mortality %
Anadol 80% WP	250 gm	2.11	3.19	12.43	17.73
Duet-M73% WP	150 gm	2.25	2.21	11.63	16.09
Index 77% WP	250 gm	2.65	3.71	13.71	20.07
Optima 25% WP	50 cm^3	3.15	4.71	16.53	24.21
Remik 30% WP	200 gm	2.71	3.66	14.75	21.12
Roxyl puls 68.9% WP	150 gm	3.17	5.82	22.37	31.36
Sandcure 72% WP	250 gm	3.22	7.13	23.42	33.77
Untreated	-				

These data are in accordance with those reported by several authors. Martimson and Williams (1996) mentioned that the field equivalent rates of fungicide, were relatively nontoxic to *A.epos* adults, the parasitoid of *Erythroneura* leafhoppers,(Homoptera: Cicadellidae) but they found that sulphur (9600ppm) elevated mortality of *A.epos* adult exposed for 14 to 21 days post treatment.

Abdelgader and Hassan (2002) showed that azoxystrobin fungicide was slightly to moderately harmful to the adults of *Trichogramma cacoeciae*

Tedeschi (2002) (Hymenoptera Trichogrammatidae) sayed that sulphur and thiophanate – methyl (TPM) were slightly and moderately harmful to the mymphal development of the predatory bug *Macrolophus caliginosus*

Wagner, 1951 (Heteropterai, Miridae) under oratory conditions, respectively, reported that fungicides sprayed on pepper plats can affect the ECLW populations on pepper plants under greenhouse conditions. Idinger (2002) mentioned that *F.condida* (Collembola) was more susceptible to Eupar en M than copper oxychloride with regarded to lethal effects. He added that reproduction rate was significantly reduced by two fungicides.

El-Kholy (2005) mentioned that the tested fungicides reduced the feeding ratio of *S.littoralis* larvae, weight of larvae and pupae, pupation % and adult emergence and also fecundity and fertility when 4th instar larvae of *S.littoralis* were fed on treated leaves with Micronised Sulphar, Dithane M45, Galbin Copper and Ridomil plus. Topas, Delcup and Rubigan were the least effective. El-Sisi (2016) indicated that beside using fungicides, they showed that slightly initial and latent effects against second and fourth instar larvae of *S.littoralis* and high developmental effect against both pupae and moth stages enough to broke the insect life-cycle especially in case of copper sulphate and copper Oxychloride against second instar larvae of this insect. Srivastava ., (2017). Reported that Ridomilm and Mancozeb fungicides affect the growth and development of *S.litura* at higher concentrations. The larval duration was significantly prolonged. Also, pupation rate, adult emergence and longevity and fecundity was reduced by these fungicides.

We concluded that, from these results, these fungicides can serve a practical tool to reduce the *S.littoralis* populations in potato fields and may assume a greater role in integrated program showed to the manage insect pests and diseases. These fungicides also can affect the first generation as latent effect of the ECLW.

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التأثيرات الجانبية لمبيدات الفطريات على دودة ورق القطن المصرية التي تصيب نباتات البطاطس

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تمت دراسة التأثيرات الجانبية لسبعة من مبيدات الفطريات المستخدمة في حقول البطاطس لمكافحة مرض الندوة المبكرة على حشرة دودة ورق القطن المصرية . تم غمر أوراق الخروع في المبيدات الفطرية على المعدلات الموصي بها في الحقل طبقاً لتوصيات وزارة الزراعة واستصلاح الأراضي لمكافحة مرض الندوة المبكرة على البطاطس. ثم دراسة معدل التغذية والموت ونمو وتطور اليرقات والعذاري والحشرات الكاملة, وكذلك تأثير هذه المعاملات على الجيل الأول الناتج من هذه المعاملات . تمت تغذية يرقات العمر الرابع لمدة 24 ساعة على ورق الخروع المعامل بالمبيدات في المعمل ثم على أوراق غير معاملة حتى التعذير. ودلت النتائج على أن هذه المركبات تنقص من معدل التغذية ووزن اليرقات ويزيد من العمر اليرقي (بالأيام) ولوحظ فروق على عدد البيض الموضوع ولكنها تنقص من عدد البيض بصورة غير معنوية، كما لوحظ أن هذه المعاملات تنقص من طول عمر الفراشات الذكور والإناث وتؤثر بصورة كبيرة على الجيل الأول الناتج .