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Fate of ^{14}C-Aldicarb in Parasitized *Spodoptera littoralis* Boisd. Larvae*

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Abstract: This study was carried out to determine the fate of ^{14}C-aldicarb in parasitized *Spodoptera littoralis* Boisd (Lep.: Noctuidae) larvae fed with ^{14}C-aldicarb treated lettuce leaves. ^{14}C-aldicarb equivalent residues were found 75.49 % , 1.14 % and 3.4 % of initially applied radioactivity in the feces of *Spodoptera littoralis and* cadaver, and parasitoid (*Chelonus oculator* Panzer Hym.:Braconidae), respectively. Results showed that aldicarb metabolized rapidly and excreted out of body by feces within 24-48 hours, before the attack of parasitoid to host organs.

Key Words: *Spodoptera littoralis*, *Chelonus oculator,* ^{14}C-aldicarb, residual toxicity

Parazitli *Spodoptera littoralis* Boisd. Larvalarında ^{14}C-Aldicarbın İzlenmesi

Öz: Bu çalışmada ^{14}C-aldicarb uygulanmış marul yaprakları ile beslenen parazitli *Spodoptera littoralis* Boisd (Lep.: Noctuidae) larvalarında aldicarbın izlenmesi amaçlanmıştır. Başlangıçta uygulanan ^{14}C-aldicarb'ın % 75.49'u *Spodoptera littoralis'* in dışkılarında, % 1.26' sı kadavrasında ve % 3.02' si parazitoidde (*Chelonus oculator* Panzer Hym.:Braconidae) bulunmuştur. Bu sonuçlar, parazitoidin konukçu organlarına saldırısından önce, 48 saat içinde aldicarb'ın hızla metabolize olduğu, dışkı ile vücuttan atıldığını göstermiştir.

Anahtar Kelimeler: :*Spodoptera littoralis*, *Chelonus oculator,* ^{14}C-aldicarb, kalıntı toksisitesi

Introduction

S. littoralis is a serious pest of cotton plantation in Turkey (Anonymous 2000). Parasitoid *C. oculator* was reported as the first record of Turkish fauna (Özkan and Özmen, 2001), some other *Chelonus* species had been reported in Beyarslan 1985. The parasitoids of genus *Chelonus* are unique in that they induce in their hosts the precocious onset of metamorphosis and developmental arrest in the precocious prepupa (Rechav and Orion, 1975; Jones, 1987; Grossniklaus-Bürgin et al. 1994). Detaied biological information is lacking on *C. oculator,* an egg-larval parasitoid of *S. littoralis.*

Aldicarb [2-methyl-2-(methylthio) propionaldehyde o-(methylcarbamoyl) oxime] is a soil applied systemic carbamate insecticide recommended for the control of chewing and sucking insects, spider mites, and nematodes in glasshouse and outdoor crops. Mode of action is systemic, with contact and stomach action. It is absorbed rapidly, through the roots with translocation acropetally. This insecticide is a cholinesterase inhibitor with toxicity of acute oral LD_{50} for rat 0.93 mg kg^{-1}. Acceptable daily intake (ADI) for aldicarb is 0.003 mg kg^{-1} body weight (Worthing and Hance 2001).

Aldicarb is registered for use in Turkey on cotton for control of *Bemisia tabaci* Genn. (Hom. Aleyrodidae) and on release from the granule (Anonymous 2001). Aldicarb sucking insects (Anonymous 2002). Aldicarb is applied at planting and disperses through the soil with soil moisture and its metabolites can persist in the soil and carry over into the following year's crops (http://www.abcbirds.org/pesticides/ Profiles/aldicarb.htm). Therefore, there is a risk of indirect aldicarb exposure for many nontarget insects that feed on treated plant leaves (Stapel et al. 2000). Egyptian cotton leafworm (*Spodoptera littoralis*) and its parasitoid *Chelonus oculator* are nontarget insects in the aldicarb used area.

A number of workers have investigated metabolism of aldicarb in plant and insect (Metcalf et al. 1966, Andrawes et al. 1971 and 1973, Tunçbilek et al. 1997), but there is a few work on effects of systemic insecticide on pest and their parasitoids (Stapel et al. 2000, Rebek and Sadof 2003).

Earlier studies showed that aldicarb metabolised in the cotton plant and in the house fly (*Musca domestica L.*) through oxidation to aldicarb sulfoxide [2-methyl-2(methylsulfinyl) propionaldehyde *O*-(methylcarbomyl)-oxime] and aldicarb sulfone[2-methyl-2(methylsulfonyl) propionaldehyde *O*-(methylcarbomyl)oxime] (Worthing and Hance 2001). The former, which is 10-20 fold active as a cholinesterase inhibitor than aldicarb itself, is the major metabolite in the foliage during early stages of plant growth and the latter, which is responsible for the persistent systemic activity of the compound, becomes the

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predominant one during maturation of the plant (Metcalf et al. 1966, Andrawes et al. 1971, Worthing and Hance 2001).

Metcalf et al. (1966), have worked on metabolism of aldicarb in plant and insect. It was found that following topical treatment of houseflies with ^{14}C-aldicarb, a large proportion of the ^{14}C is liberated in the feces over 24 hours. The percentage of the absorbed dosage thus excreted varied with the total dosage and with the fly strain, being much higher at lower dosages and with resistant flies. However, where the flies survived over the 24-hour period, from 40 to 60% of the total dosages was excreted in the feces.

Stapel et al. (2000), reported that host foraging ability and longevity of the parasitoid *Microplitis croceipes* Cresson reduced after feeding on extrafloral nectar from coton plants which were treated with systemic insecticides. The insecticides used in the study are regularly applied in cotton-growing areas in the United States. Longevity of *M. croceipes* females that fed on nectar from cotton was affected for at least 10 days after plants were treated with insecticides. Moreover, the parasitoid's host foraging ability was severely affected for periods ranging from 2 days (imidacloprid) to 18 days (aldicarb) after the insecticide application. The consequences of these sublethal effects on the success of biological control were discussed. Therefore, they predicted that certain systemic insecticides might depress the impact of parasitoids attacking lepidopteran pests in cotton.

Langley and Stark (1996), worked on laboratory bioassays designed to quantify the direct, residual and oral exposures of the aphid parasitoid *Aphidius ervi* Haliday to a diazinon, using gas chromatography and radiotracer technique. Researchers demonstrated that the use of radiolabelled diazinon samples enabled greater, or more precise, detection of quantities of ingested active ingredient per unit time of feeding. With isotope aided studies radioactivity is measured irrespective of the structure of the compound, while gas chromatography analysis only measures parent compound.

A number of research have focused on to determine the lethal effect of insecticides or its residues to adult parasitoids (Erol and Kılınçer 1986, Yiğit and Uygun 1986, Kılınçer et al. 1990, Stansly and Liu 1997, Delpuech et al. 1998, Jones et al. 1998, Hill and Foster 2000, Tillman and Mulrooney 2000, Brunner et al. 2001, Consoli et al. 2001, Takada et al. 2001, Haseeb and Amano 2002, Tang et al. 2002, Kramarz and Stark 2003, Langhof et al. 2003, Symington 2003, Williams III et al. 2003). Although studies, such as effect of insecticides on immature stage of parasitoids via trophic interactions are also examined (Butaye and Degheele 1995, Iqbal and Wright 1996, Floate and Fox 1999, Erb et al. 2001), a few of them are related to systemic insecticides. (Stapel et al. 2000, Rebek and Sadof 2003).

For the reasons mentioned above, we aimed to determine effect of aldicarb residues on one of the non-target insects *S. littoralis* and its parasitoid *C. oculator* in the laboratory conditions. For this aim, 7 mg kg^{-1} residue level, which is found on cotton leaves 100 days after planting, is used for the application dose (Andrawes et al. 1971). Moreover the most harmful period of *S. littoralis* in cotton fields is 105-110 days after planting (Anonymous 2000).

Materials and Methods

Chemicals: Radiolabelled aldicarb (6.06 mCi mmol^{-1}) was supplied by Rhone-Poulenc Com. Unlabelled and radiolabelled aldicarb were mixed and radioassayed by direct liquid scintillation counting to determine the actual concentration of the compound in the formulation. The specific activity of the solutions were 104.64 Bq μg^{-1} (6280 dpm μg^{-1}, corresponding 7 mg kg^{-1}) and 92.7 Bq μg^{-1} (5562 dpm μg^{-1}, corresponding 7 mg kg^{-1}) for first and second experiment, respectively. Two point five microliter (which is equal to 7656 dpm and 6952 dpm for the first and second experiment, respectively) of this solution was applied to dorsal surface of 174 mg lettuce leaf disk, with Hamilton syringe. First and second experiments were carried out with fifteen and ten parasitized larvae, respectively.

Radioactivity was determined by using a Packard Tricarb 1550 Liquid Scintillation Analyser. The solution for liquid scintillation counting was Hionic Flour (Packard 6013319). Half milliliter of tissue solubilizer (Soluene 350) was added to the samples placed on combusting boat. Samples were combusted in a Harvey Biological Oxidizer, OX-600. The cocktail used for trapping $^{14}CO_2$ from combusted samples was prepared by mixing cocktail (50 mg POPOP +5 mg PPO in 1 liter toluene) and absorber (125 ml ethanolamine in 875 ml methanol) at the rate of 1:1 in methanol (IAEA, 1991).

Rearing techniques: The *Chelonus oculator* Panzer (Hym.:Braconidae) colony and *Spodoptera littoralis* Boisd (Lep.: Noctuidae) eggs were collected from Çukurova region in Turkey. Parasitoid colony was obtained from parasitized *S. littoralis*. Parasitoid was identified by Dr. M. Shaw (National Museum of Scothland, Department of Geology and Zoology, Edinburgh, England). The *C. oculator* colony was maintained in an insectary under 27±1 ^{o}C and photoperiod of 14:10 (L:D) h using *Spodoptera littoralis* as the host. *S. littoralis* were reared in an insectary as described in Patel and Patel (1971) and its diet was chosen based on Abdel-Fattah et al (1977).

Ten female-male pairs of adult parasitoids were housed in a plastic container. Nourishment was provided by a thin layer of honey at the bottom of the container. One day old *S. littoralis* eggs were used for parasitization. A paper sheet containing 9-10 *S. littoralis* egg patches (400-700 eggs) was placed in the container for 24 h. Then they were placed into petri dishes (9 cm diameter) for incubation.

Parasitized *S. littoralis* larvae were placed on lettuce leaves immediately after the eggs hatched and kept up to third instar (5-6 day old) in diet container. Each parasitized *S. littoralis* larvae were placed into small petri dishes (5 cm diameter) and reared on lettuce leaves separately. Under

these rearing conditions parasitized larvae enter precocious metamorphosis in the fifth instar and parasitoids emerge from the host 12-13 days after oviposition. They feed on the host, spin a cocoon and pupate in the cocoon of the host, where they remain for 7-8 d until the adults emerge.

Experiment: To determine the fate of insecticide, 10 day old parasitised *S. littoralis* larvae were taken from the laboratory-reared population. ^{14}C-aldicarb was applied to lettuce leaf. Parasitized *S. littoralis* larvae were placed onto treated leaf for 24 h for feeding, then the ones, consumpted whole leaf disk, were selected (Figure 1). Further detail for laboratory experiment, combustion of samples, and LSC analysis of ^{14}C-aldicarb are shown in Figure 1.

To find out fate of ^{14}C-Aldicarb in parasitized *S. littoralis* larvae two experiments were carried out. In the first experiment only the cadaver of *S. littoralis* and parasitoid *C. oculator* were assayed with the 15 replication. Second experiment was also included feces of *S. littoralis* larvae with the 10 replication.

Results and discussions: The percent of ^{14}C-aldicarb equivalent residues were shown in Table 1. Since the results for the first experiment in the table indicated low recovered radioactivity in the parasitoid and cadaver, feces of *S. littoralis* larvae were also included in the second experiment. It was found that *S. littoralis* and its parasitoid were not affected by aldicarb residues even 10 fold of residue doses. A huge amount of applied aldicarb was found in host feces as shown in Table 1. Although parasitoids emerged and fed on host externally, only 3.02% of initially applied aldicarb was found in their body. These results indicate that aldicarb was excreted from *S. littoralis* body by feces within 48 hours, before the attack of parasitoid to host organs.

Detailed results are illustrated in Figure 2. Excreted ^{14}C-aldicarb in the feces ranged from 64.65 to 95.65 % of applied radioactivity. Similar results have been reported earlier indicating Temik was excreted in the feces from 40 to 60% of the total ^{14}C-Temik (Metcalf et al. 1966). Previous study has demonstrated that ^{14}C-Chlorpyrifos excereted about 50.93% of applied radioactivity by the feces in resistant *S. littoralis* (Özyardımcı 2002). These findings are compatible with Neumann and Guyer (1987)'s comment indicating rapid metabolisation and excretion of some insecticides resulting in resistancy in the insect against the insecticides. The results obtained in this study show that 75.49 % of aldicarb localized in the feces.

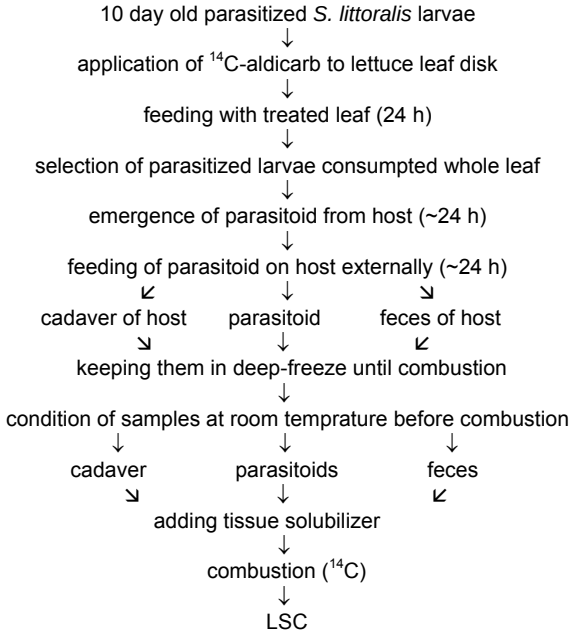

Figure 1. Schematic diagram of experiment and analysis of ^{14}C-aldicarb

Reports dealing with systemic insecticide effects on non-target insects especially parasitoids and their hosts are scarce. Although systemic insecticides are claimed to be fairly safe for beneficials lack of direct exposure, Stapel et al. (2000) showed that acephate, imidacloprid and aldicarb can contaminate nectar and through feeding on treated cotton, adult parasitoids were affected. Rebek and Sadof (2003) suggested that impact of sublethal doses of systemic insecticides on parasitoids are more toxic than their hosts.

As a result of this laboratory study it was found that *S. littoralis* and its parasitoids were not affected the residue of aldicarb when the host population reach to the most harmless level in the field. This will make possible to use of parasitoid in the aldicarb treated area.

Conclusion: The use of ^{14}C-labelled pesticide is a very useful tool for revealing the fate of pesticide in the insect, plant and environment; without any chromatographic techniques. With this study it was obvious that 75 % of applied ^{14}C-aldicarb excreted by feces within 48 h after application. This result was only sensitively attainable using radiotracer techniques. On the other hand, the identification of ^{14}C-related residues (i.e. main compoundand/or its metabolite) is also very important. Therefore, to carry out experiment with large

Table 1. Distribution of ^{14}C-aldicarb in the parasitized *S. littoralis* larvae fed with treated lettuce leaf*

Sample	First experiment		Second experiment		Average of applied radioactivity (%)
	dpm	% of applied radioactivity **	dpm	% of applied radioactivity ***	
Parasitoid	203.2	2.65	236.7	3.40	3.02
Cadaver	106.5	1.39	79.87	1.14	1.26
Feces	-	-	5248.6	75.49	75.49

* Average values of 15 and 10 replicates for the first and second experiment, respectively
** Applied radioactivity : 7656 dpm leaf^{-1} disk
*** Applied radioactivity : 6952 dpm leaf^{-1} disk

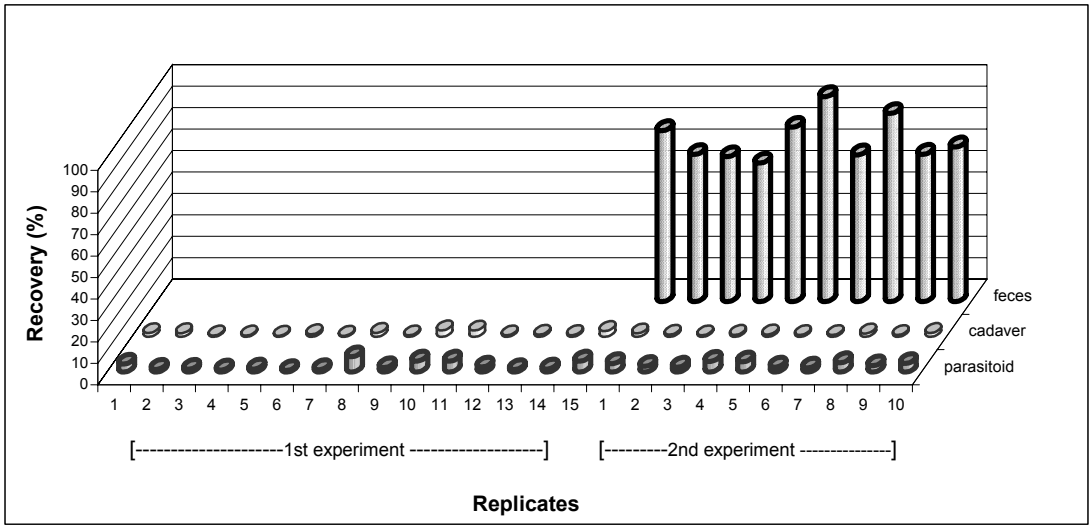

Figure 2. Recovered radioactivity individual replication

amount of insect and analyze them with the combination of radiotracer and chromatographic techniques is necessary and this can give hints for the future investigations.

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