

Laboratory Evaluation of Tween 20 for Potential Use in Control of *Cacopsylla pyri* L. Eggs and Nymphs (Homoptera: Psyllidae)

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ABSTRACT

The toxicity of the Tween 20, also known as polyoxyethylene (20) sorbitan monolaurate, was evaluated against the summerform pear psylla *Cacopsylla pyri* (L.) (Homoptera: Psyllidae) under laboratory conditions. Tween 20 was applied at 1, 2, 3, 7, 10, 20 and 30 ml/l concentrations onto eggs and nymphs of *C. pyri* on pear shoots. Water was used as a control treatment. The toxic effect on eggs and nymphs was determined by counting treated eggs and nymphs, live or dead. Despite low toxicity to psyllid eggs, Tween 20 showed significant toxic effects against nymphs. The mortality rates of eggs were 3.2%, 2.1%, 1.0%, 12.8%, 13.5%, 100.0%, 100.0% at 1, 2, 3, 7, 10, 20 and 30 ml/l concentrations, respectively. The highest toxic activity was recorded at concentrations of 20 and 30 ml/l. The mortality rates of nymphs were 7.2%, 21.0%, 49.9%, 96.5%, 100.0%, 96.5%, and 100.0% at above concentrations in the same order, respectively. Tween 20 at 3, 7, 10, 20 and 30 ml/l concentrations showed the highest toxic activity to psyllid nymphs, while concentrations of 1 and 2 ml/l had the lowest toxicity compared to water control. No significant phytotoxic effect was observed at all concentrations except for concentrations at 20 and 30 ml/l. In conclusion, Tween 20, either alone or in combination with pesticides, may provide an effective alternative to control pear psylla nymphs.

Keywords: Tween 20, *Cacopsylla pyri*, Toxicity, Detergents, Soaps, Surfactants, Pear

INTRODUCTION

Pear [*Pyrus communis* L. (Rosaceae: Rosales)] is grown widely throughout Turkey. Approximately 460.000 tonnes of pears were produced in 2013 throughout the country. Turkey is the second-largest pear producer in Europe and ranks fifth in pear production in the world (FAO 2013a). However, pear exports remain low with 12.585 tonnes compared to the total production in 2013 (FAO 2013b). Also, pear exports to European Union have slowed down recently due to undesirable pesticide residues in the fruit. This is because growers are heavily reliant on pesticide use to control pear psylla [*Cacopsylla pyri* L. (Homoptera: Psyllidae)]. There is a great need to reduce reliance on pesticides by adopting alternative control methods to chemical control of this key pest of pears (Erler 2004, Silva *et al.* 2005, Sanchez and Angula 2012).

C. pyri adults and nymphs cause damage by feeding on shoots and leaves, producing honeydew, thus reducing the photosynthesis and forming black surface on fruits (Stamenkovic *et al.* 2001, Blomquist and Kirkpatrick 2002, Garcia-Chapa *et al.* 2005, Sule *et al.* 2007). Pear psylla infestation may become widespread if control practices are not implemented in the early season. Plants infested with psylla exhibit reduced fruit quality, especially when young fruits are covered by sticky psylla honeydew. This indirect damage results in the loss of market value of mature fruits. In recent years, chemical control of pear psylla has also become more difficult because of pesticide resistance (Weissling *et al.* 1997). Since chemical control of this pest is currently more troublesome, more expensive and less trustworthy, alternative control methods to pesticides are necessary for management of pear psylla (Erler and Cetin 2007).

Detergents, soaps, and surfactants may provide safer alternatives to pesticides for controlling insects. In fact, they have a different mode of action compared with pesticides. The insecticidal effect of soaps and detergents against insects and mites may be caused by several mechanisms such as the inhibition of enzymatic processes, the destruction of biological membranes, the removal of waxes from arthropod cuticle, the knockdown of pests from the foliage, and the penetration of soaps and detergents into tracheae or combinations of all (Tipping *et al.* 2003). Detergents and surfactants can be used as wetting, spreading, emulsifying and sticking agents to improve the effectiveness and coverage of many pesticide formulations (Liu and Stansly 2000). Detergents may be applied directly to control soft-bodied pests (Green and Chou 1993) including aphids,

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mites (Lawson and Weires 1991), thrips (Warnock and Loughner 2002), whiteflies (Nava-Camberos *et al.* 2001) and mealybugs (Ware 1994).

C. pyri gives 3-4 generations each year in Bursa (Kovanci *et al.* 2000). The high reproductive ability of this pest may be responsible for substantial economic losses. Pesticide resistance build-up in pear psylla populations, as well as pesticide residue problems in fruits, emphasize the need for more reliable compounds for pear psylla control. Moreover, increased number of sprays may also lead to the destruction of the natural enemies such as *Anthocoris nemoralis* (F.) (Gencer and Kovanci 2000). In contrast, detergents and soaps have less impact on the natural enemies and environment (Curkovic and Araya 2004). Besides, they are more cost-effective than pesticides.

The aim of this study was to evaluate the insecticidal effect of Tween 20 surfactant against *C. pyri* eggs and nymphs.

MATERIALS AND METHODS

Experiments were carried out in the Entomology Laboratory at Department of Plant Protection in Uludağ University, Turkey in 2015. Treatments were composed of Tween 20[®] at 1, 2, 3, 7, 10, 20 and 30 ml/l concentrations and water control. Summerform adults of pear psylla were collected by insect aspirator from untreated pear orchards in İnegöl town of Bursa. Firstly, adults were transferred to pear shoots and placed in transparent cages at room temperature of 24 °C. They were subjected to a long-day photoperiod of 14 hour light and 10 hours dark for the experiments. Tween 20 and water mixture were applied by hand sprayer. There were three replicates for ovicidal and nymphicidal assays with pear psylla.

Toxicity of Tween 20 to *C. pyri* Eggs

The toxic effect of Tween 20 on eggs of summerform females was studied. Pear shoots, which were collected from pear seedlings, were shortened 25 cm and then placed in transparent cages one by one. Five adult females were put into each cage, and the number of eggs on each shoot was recorded after 72 hours and females were removed from the cages. Tween 20 concentrations and water for control applied to the eggs on the shoots. The toxic effect of Tween 20 on eggs was determined by counting live or dead (wrinkled and dark brown) eggs on sprayed shoots 7 days after the treatment.

Toxicity of Tween 20 to *C. pyri* Nymphs

The nymphicidal effect of the Tween 20 was also examined against 1st and 2nd stages of pear psylla nymphs. Pear shoots at 25 cm length were placed in transparent cages one by one and then five summerform females were put into each cage. Adult females were removed from the cages after 72 hours. Following the hatching of eggs, the number of eggs was recorded on each shoot. Different Tween 20 concentrations and water control were applied to the 1st and 2nd stage nymphs on the shoots. Live or dead nymphs on sprayed shoots 3 days after the treatment were counted to assess the toxic effect of Tween 20 on nymphs.

Statistical Analysis

Mortality data were converted to Abbott's corrected formula, and mortalities in the controls had been removed from the mortality in the each treatment to adjust mortality data. Ovicidal and nymphicidal toxic effects of Tween 20 on summerform pear psylla were evaluated by the analysis of variance (ANOVA). If there were significant interaction effects between Tween 20 and control treatment, LSMEANS comparisons were used to identify these effects. Fisher's protected LSD test was used to compare mortality means ($P=0.05$).

Phytotoxic Effects of Tween 20

Once Tween 20 applications were made on shoots, the phytotoxic effects of different concentrations on plant parts were examined using specific damage criteria for deformation on leaves and shoots such as discoloration, necrosis and lesions.

RESULTS

Ovicidal Effect

The toxicity of Tween 20 at various concentrations to *C. pyri* eggs is shown in Table 1. In laboratory bioassays, the survival rates of eggs were significantly different among all the tested concentrations and the controls 7 days after the treatment ($F_{7,16}=12.9$, $P>0.0001$). Tween 20 at 1, 2, 3, 7, 10, 20 and 30 ml/l concentrations resulted in 3.2%, 2.1%, 1%, 12.8%, 13.5%, 100.0%, 100.0% mortality to *C. pyri* eggs, respectively. At concentrations of 20 and 30 ml/l the highest toxic activity was recorded (Table 1). No significant differences were observed between concentrations of 1, 2, 3, 7 and 10 ml/l concentrations as compared to control.

Nymphicidal Effect

The toxicity of Tween 20 at various concentrations to *C. pyri* nymphs is shown in Table 2. In laboratory bioassays, the survival rates of nymphs were significantly different among all the tested concentrations and the controls 3 days after the treatment ($F_{7,16}=16.9$, $P>0.0001$). Tween 20 at 1, 2, 3, 7, 10, 20 and 30 ml/l concentrations resulted in 7.2%, 21.0%, 49.9%, 96.5%, 100.0%, 96.5%, 100.0% mortality to *C. pyri* nymphs, respectively. The highest toxic activity was recorded at concentrations of 3, 7, 10, 20 and 30 ml/l (Table 2). No significant differences were observed between the lowest concentration of Tween 20 at 1 ml/l and water control.

Table 1. The efficacy of various concentrations of Tween 20 on eggs laid by summerform *Cacopsylla pyri* females, 7 days after treatment.

Material	Egg mortality (%) (\pm SH)						
	Concentrations (ml/l)						
	1	2	3	7	10	20	30
Tween 20	3.2 \pm 2.9 b	2.1 \pm 2.1 b	1.0 \pm 1.0 b	12.8 \pm 7.6 b	13.5 \pm 11.5 b	72.8 \pm 11.4 a	100.0 \pm 0.0 a
Water (Control)	0.0 \pm 0.0 b						
Statistic	$F_{7,16}=12.9$, $P>0.0001^*$						

Same letter are not significantly different at the 5% level of probability using the LSD test.

*The mortality rates of eggs were significantly different among all the tested concentrations and the controls 7 days after the treatment

Table 2. The efficacy of various concentrations of Tween 20 on nymphs of *Cacopsylla pyri*, 3 days after treatment.

Material	Nymph mortality (%) (\pm SH)						
	Concentrations (ml/l)						
	1	2	3	7	10	20	30
Tween 20	7.2 \pm 7.2 b	21.0 \pm 16.9 b	49.9 \pm 7.2 a	96.5 \pm 3.5 a	100.0 \pm 0.0 a	96.5 \pm 1.9 a	100.0 \pm 0.0 a
Water (Control)	0.0 \pm 0.0 b						
Statistic	$F_{7,16}=16.9$, $P>0.0001^*$						

Same letter are not significantly different at the 5% level of probability using the LSD test.

*The mortality rates of nymphs were significantly different among all the tested concentrations and the controls 3 days after the treatment

Phytotoxic Effect

No significant phytotoxic effect was observed on pear leaves and shoots at all concentrations except for concentrations at 20 and 30 ml/l.

DISCUSSION

Tween 20 at 1, 2, 3, 7 and 10 ml/l concentrations showed the lowest toxic activity to *C. pyri* eggs while 20 and 30 ml/l concentrations had the highest toxic activity. On the other hand, the highest toxic activity to psyllid nymphs was obtained with Tween 20 at 3, 7, 10, 20 and 30 ml/l concentrations but concentrations of 1 and 2 ml/l caused the least toxicity compared to water control. No significant phytotoxic effect was observed at all concentrations except for those at 20 and 30 ml/l water.

As regards to the toxicity of Tween 20 to pear psyllid nymphs, our results are in agreement of those of Panahi *et al.* (2013), who obtained 60-93%, 66-96%, 61-82%, 55-73% mortality 2, 7, 14, 21 day after the

treatment of liquid detergent, respectively, for control of common pistachio psylla, *Agonoscena pistaciae* nymphs. Toxic effects of detergents on various pests have been investigated vigorously in previous studies. Puri *et al.* (1991) used washing powder detergent (Nirma) to control sweet potato adult whiteflies *Bemisia tabaci* Genn. Treatments were reported to be effective from 1 day to 7 days after treatment at 30 ml/l dose in field studies. Besides, the numbers of nymphs were found to be reduced by the treatment of individual leaves with washing powder at 1.5, 15 and 30 ml/l concentrations. According to their study, there was little phytotoxicity observed when individual leaves of cotton cultivars were sprayed with washing powder at 15 and 30 ml/l concentrations. Vavrina *et al.* (1995) applied detergents of sodium dodecyl benzene sulphonate and sodium lauryl ether sulphate to control *B. argentifolii* Bellows & Perring nymphs in tomatoes. Phytotoxic effects of detergents were observed at 2 and 3 ml/l concentrations on leaves and shoots in laboratory experiments as we also detected in our study.

Curcovic and Araya (2004) sprayed two detergents (Nobla and Quix) against *Panonychus citri* and *P. ulmi* in laboratory experiments. Based on their findings, the mortality rates of mites ranged between 32% and 91% at 0.25% concentration of Quix and 0.45% concentration of Nobla, respectively, compared with control (22%). Curcovic *et al.* (2006) had evaluated the pesticide activity of the agricultural detergents SU-120 and Tecs Fruta on *Tetranychus urticae* Koch and *Myzus persicae* (Sulzer). Researchers reported near 70% mortality on *M. persicae* adults and nymphs when they applied SU-120 at 2.5%. Their findings also indicated that Tecs Fruta at $\geq 5\%$ solution yielded $\geq 85\%$ mortality of adults while it had $>70\%$ mortality on nymphs at $\geq 2.5\%$ solution.

Tippling *et al.* (2003) examined the efficacy of an organosilicone surfactant against various pests of table grapes. Silwet L-77 at 0.1% concentration was found to be highly toxic (99.4% mortality) to *T. pacificus* McGregor eggs. Furthermore, 0.1, 0.25, and 0.5% concentrations of Silwet L-77 had shown 93.8%, 98.5%, and 99.4% toxicity to cotton aphid (*Aphis gossypii* G.), flower thrips (*Frankliniella occidentalis* P.), and Pacific spider mite (*T. pacificus*) immature and adult stages, respectively. Authors also added that Silwet L-77 at 0.5% and 1% concentrations provided 100% mortality against grape mealybug, although percent mortality was only about 6.7% when only 0.1% concentration was applied.

Butler *et al.* (1991) applied laundry detergent powder (Nirma) to control sweetpotato whitefly, *B. tabaci* (G.) nymphs. They managed to kill 96% of the whole population 5 days after treatment. The treatments also reduced the number of cotton aphids, *A. gossypii* at an average mortality rate of 87-95%. As we found in our study, detergents appeared to be very effective, especially against the immature stages of Homopteran pests in various studies.

As a result, Tween 20 treatments proved to be toxic against pear psyllid eggs and nymphs. Tween 20 treatments at 3, 7, and 10 ml/l concentrations successfully controlled first and second stage nymphs of psylla without causing any phytotoxicity. However, it is important to note that there is a direct relationship between Tween 20 concentration and mortality. Apparently, Tween 20 may provide an effective alternative to control pear psylla eggs and particularly nymphs. Further field trials are required to confirm the results of laboratory studies.

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