

EFFECT OF RICINUS COMMUNIS EXTRACTS ON WEIGHT AND MORTALITY OF SCYPHOPHORUS ACUPUNCTATUS (COLEOPTERA: CURCULIONIDAE)

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Abstract

The agave snout weevil *Scyphophorus acupunctatus* is considered the major insect pest of the tequila agave *Agave tequilana* Weber var. azul and other agavaceae. The effect of hydroethanolic extracts from leaves and seed of wild (WV) and Mirante variety (MV) castor bean *Ricinus communis* L. on adults mortality and weight was assessed. When extracts were applied on agave tissue, mortality was not significant at concentrations of 0.1 to 100,000 ppm and at the tested frequencies. In the acute application, extracts from MV leaves and seed caused the largest weight loss. Within the second application, insect weight decreased even more with all of the extracts tested, however, this did not occur with the third application of the extracts where weight gains and losses of adult weevils were recorded. It is concluded that these extracts do not have toxic activity on adult weevils, but they do have an effect on weevil weight.

Key words: Extracts, mortality, weight variation

1. INTRODUCTION

The agave snout weevil *Scyphophorus acupunctatus* Gyllenhal (Coleoptera: Curculionidae) is a cosmopolitan insect considered the major pest of agaves. The larvae bore the stem and leaves and the adult lays eggs on the plant. Damage caused by the weevils also favors the presence of *Erwinia cativida*, *Pantoea agglomerans* and *Pseudomonas* sp. (Jiménez et al., 2004), which may kill the agave plant (Aquino-Bolaños et al., 2007). Several methods have been used to control the agave snout weevil been the most common the application of Tebuprimphos® and Terbufos® (De Liñán, 2009), which are harmful to growers' health, not very effective and not ecologically sound. Therefore, it is important to implement effective control actions with acceptable ecological, economic and social results.

Extracts from leaves and seed of castor bean *Ricinus communis* L. (Euphorbiaceae) have been used successfully in the management of curculionids of agricultural importance (Niber, 1994; Tinzaara et al., 2006). They cause death by ingestion and contact (Calle et al., 1996), may repel the insects or have insectistatic properties (Rodríguez, 2005). The extracts made with water, ethanol, methanol, dichloromethane, petroleum ether and hexane (Rodríguez, 2005) have been shown to have biological activity against insects (Upasani et al., 2003; Mushobozy et al., 2009).

This article reports the effect of hydroethanolic extracts from wild and Mirante variety castor bean, *R. communis* L., on weight and mortality of adult agave snout weevil *S. acupunctatus* at different concentrations by acute applications, chronic exposure and ingestion.

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2.- MATERIALS AND METHODS

2.1 Insects.

Adult agave snout weevils were collected from March 2008 to May 2009 in commercial plantations of tequila agave *A. tequilana* located in at Yautepec, Morelos (1,201 masl). Agave cores (6-7-years old) were dissected to obtain adult weevils. Insects were placed in 1 L plastic containers with perforated lids to allow air flow. The weevils were fed pieces of the agave core or the bases of the leaves of the tequila agave (250 g approximately per container) obtained in the same sites.

The collected adults were sexed at the lab, using a stereoscopic microscope (Nova[®], model ST623, magnified 4x) following Ramirez's (1993) methodology. Adults males and females were kept in a bioclimatic chamber (Precision Scientific[®], model 818) at 26 ± 1 °C and $50 \pm 10\%$ RH, with a 12:12 h light:dark period. Food was changed every two weeks. Insect identification was confirmed by Dr. Hector González-Hernández of the Department of Entomology of the Plant Health Institute of the Colegio de Postgraduados, Campus Montecillos, México, from a sample of 20 adult specimens (10♀ and 10♂).

2.2 Plant Material

Seeds and leaves of wild castor beans and of the variety Mirante L. viala cahui 2007 were used. The seeds of the Mirante variety were obtained from the seed company Ceres Internacional de Semilla S.A. de C.V. These were grown during October 2008 to January 2009 to obtain leaves and mature fruits free of damage. The wild plant material was collected along the Yautepec-Cuautla highway in the municipality of Yautepec, Morelos, during the same period and with the same characteristics as those of the Mirante variety. The plant material was identified by Juan Carlos Juárez-Delgado of the Herbarium of the Universidad Autónoma del Estado de Morelos (HUMO), where a voucher specimen (registration number 27002) was deposited.

Fruits and leaves were dried in the shade. Fruits were broken manually to extract the seeds. The leaves were shredded in a hand mill (Estrella[®], model 41B) and an electric mill (Moulinex[®]) and sifted through a number 30 mesh to obtain a fine powder. The seeds were ground in an industrial mill (Siemens Pulvex Plastic[®]) where an oily paste was obtained. The leaf powder and seed pastes were placed in amber colored glass jars (1 L) and in polyethylene bags, respectively, and left in a cool, dry place protected from light.

2.3 Hydroethanolic Extracts

One kilogram of leaf powder and 1 kg of oily seed paste were macerated for 24 h in 2.5 and 4.5 L of ethanol and distilled water (70:30) and placed in glass flasks of 5 and 20 L, respectively. The macerate was filtered through cotton gauzes in a plastic funnel into a 3 L Erlenmeyer flask and concentrated in a rotavapor (Büchi R-114) at 55°C and 30 rpm.

The extracts obtained were frozen for 15 min at -68 °C in a freezer (Thermo Electron Corporation[®] ULT1783-3-A40) and lyophilized (Heto Drywinner[®] DW-3) for 24 h, obtaining fine crystals and oily pastes from the crude extracts of leaves and seeds, respectively. These materials were placed in 0.5 L amber colored jars and kept under refrigeration at -4 °C.

2.4.-Characterization of Compounds

To determine the chemical components of the hydroethanolic extracts, the plant-solvent material (1 g / 10 mL) was mixed for 24 h and concentrated in the rotovapor. The chemical components of these extracts were compared with the hexanic and methanolic extracts from leaves and seeds of the wild and Mirante variety plants. Detection of essential oils, fatty acids, terpenes, alkaloids and flavonoids were carry out by fine layer chromatography using silica gel plates (60 F₂₅₄ Merck[®], 6 x 4 cm). To reveal fatty acids, essential oils and terpenes, the ethyl hexane/acetate mobile phase (7:3) was used, and the developers were Erlich, phosphomolibdic acid and ceric sulfate, respectively. To reveal alkaloids and flavonoids, Dragendorff's developer and 2-aminoethyl-diphenylborinate, respectively, were used. We used quercetin and quercetrin as standards for comparison.

2.5 Treatments.

A 10% parent solution was prepared for each of the extracts (0.5 g / 5 mL of lyophilized extract – acetone). From this solution, serial dilutions were prepared at 10, 1, 0.1, 0.01, 0.001 and 0.0001 % (100,000, 10,000, 1,000, 100, 10, 1 and 0.1 ppm), following Lagunes-Tejeda and Vázquez-Navarro (1994) methodology.

We evaluate the hydroethanolic extracts from (1) wild castor oil bean seeds (WS), (2) Mirante castor bean variety seeds (MS), (3) wild castor bean leaf (WL), and (4) Mirante castor bean variety leaf (ML), and (5) 95% acetone as control.

2.6 Bioassays

Bioassays were conducted in the laboratory where the effect of hydroethanolic extracts applied by different methods on female and male adult agave snout weevils was evaluated: by contact (acute), residual (chronic), and ingestion.

2.7 Mortality by Contact (acute)

Adults were weighted on an analytical balance (Explorer OHAUS, EO2140, USA) and initial weight was recorded. The four treatments comprising the seven concentrations described and their respective controls (95% acetone) were applied on a population of 350 weevils (175 ♀ and 175 ♂), ten weevils per treatment (5♀ and 5♂), including the control. With a 10 µL micropipette (Rainin®), 1 µL of the extract concentrations was applied directly to the intersegmental union between the insect's pronotum and the elytra.

The treatments were applied to all of the adult insects under study, and a second application 15 d later on a random third of the original population. A third application was performed on the third of the population that received the second application 22 d after the first application. The treated insects were placed individually in number 4A plastic cups used for gelatin (6 x 4.5 cm diameter x height, REYMA ®) and kept at 27 ± 2 °C and 35% RH with a piece of tequila agave 1 x 2 cm diameter x length, which was changed every week.

The number of dead insects was recorded 1, 4, 8, 12, 24, 48, 72, 96, 120, 144 and 168 h after each application. Individuals were considered dead when there was no movement in response to stimulation with entomological pincers in its face. Live adult insects that had been subjected to the first application were weighed 15, 22 and 30 d later on the analytical balance mentioned above. The live adult insects that had received a second and third application were weighed 7, 15 and 22 d after each application. With the data obtained, percentage of mortality was estimated with respect to the total population of insects evaluated in this bioassay. The variation in weight of the insects was estimated as percent of their initial weight.

2.8 Residual Effect (chronic)

On a 5.4 cm diameter filter paper (No. 1 Whatman®), 0.5 mL (100,000 ppm) of the extract were placed and allowed to dry out for 20 min at room temperature and later placed in plastic Petri dishes (5.5 x 1.5 cm diameter x height). 95% acetone was used as control. Groups of five weevils of the same sex were placed in each Petri dish. All Petri dishes were sealed with adhesive tape to prevent the weevils from escaping. The insects were kept at 30 ± 1 °C and 43% R.H. under the same conditions as those being tested for effect by contact. The number of dead individuals was recorded for each treatment. Sixty adult insects (30♂ and 30♀) per treatment were tested. Observations were done 1, 4, 6, 8, 12, 24, 48 and 72 h after placement in the Petri dishes.

2.9 Mortality by Ingestion

Eighty adults (40♂ and 40♀) were assigned to each treatment; each individual was considered a replication. Each piece of agave (1 x 1 x 2 cm) was submerged for 4 s in a concentration of 10,000 ppm of extract, following the methodology proposed by Bogorni and Vendramim (2003). As a control, 95% acetone was used.

The treated agave pieces were allowed to dry out for 15 min on filter paper and later placed in plastic gelatin cups, described above, and five adult weevils of the same sex were placed in each one. The insects were kept at 27 ± 2 °C and 35% R.H. Mortality was assessed 1, 4, 8, 12, 24, 48 and 72 h after application.

3.- Results

3.1 Characterization of Compounds.

The analysis by fine layer chromatography (CC) to determine the chemical compounds of the hydroethanolic extracts from seeds and leaves of wild and Mirante variety *R. communis* is illustrated in Table 1. The essential oils are found in all of the seed and leaf extracts from both varieties. Fatty acids were detected in the seeds of the two varieties. Terpenes were found in the leaf extract of the Mirante variety, and finally, alkaloids and flavonoids were found in the leaf extracts of the wild and Mirante variety. With these comparisons, it is confirmed that the hydroethanolic extracts contain non-polar and polar compounds.

Moreover, the plates comparing flavonoid standards and the chemical constituents of the hydroethanolic extracts of wild and Mirante variety castor bean leaves determined the presence of rutin, quercetin and quercetrin in these extracts.

3.2 Mortality by Contact (acute).

Low or no mortality was recorded 1, 4, 8, 12, 24, 48, 72, 96, 120, 144, and 168 h after the treatment, so accumulative mortality is reported at 168 h. None of the treatments caused significant female mortality and a 4.5% accumulative mortality were achieved with the first acute application (N=175). The highest mortality was produced by LS at 1,000 ppm. No mortality was recorded at treatments SW, SM, LW and LM below 10 ppm. No mortality was recorded at LM treatment (Table 2). Male accumulative mortality for the first application was 10.28% (N=175). Mortality was recorded for all treatments; however, control accounted for the highest mortality (2.28%) (Table 3).

Accumulative mortality of females for the second application was 5.68%, with no casualties for control. The highest mortality (2.27%) was observed in the SW treatment at 0.1 ppm (Table 4). A 1.14% male mortality was obtained for SW at 100 ppm, the same as control (Table 5). For the third application, 4% of female accumulative mortality was recorded. The highest mortality (2%) was observed in treatment SW and LM at 0.1 and 10 ppm, respectively (Table 6). In this application, SM and LW treatments at 1 ppm achieved 3.8% accumulative mortality in males. No mortality was recorded for either sex.

3.3 Residual Effect (chronic)

No female or male mortality was obtained at 100,000 ppm after 72 h.

3.4 Mortality by Ingestion

Accumulative female mortality 7 d after application was 2.5%. LW extract caused no female mortality while control was 1% (table 7). For males, accumulative mortality 7 d after application was 4%; highest mortality was recorded for SM and no mortality was recorded for the control group (table 8).

3.5 Variation in Weight (acute application)

Results presented on weight variation of the agave snout weevils refer to both females and males.

3.5.1 First application. Fifteen days after the first application, SW treatment at 0.1, 1, 10, 1,000, 10,000, and 100,000 ppm did not decrease initial weight more than 2% (Figure 1 SW), however an 8% weight loss was observed at 10 ppm (Figure 1 SM). Almost all insects that were exposed to the LM extract had lower weight than those of the control group. Insects receiving LM extract at 1,000 ppm weighed 2.6, 4.6 and 7% less than their initial weight 15, 22, and 30 days after the first application, respectively (Figure 1 LM). LW treatment had no specific effect on the weight of the weevils as treated individuals either lost or gained weight (Figure 1 LW).

Twenty-two days after the first application weight gain was observed in all of the treatments with most of the concentrations, except at 1,000 ppm of LM extract where insects loss weight up to 4.6% of initial weight, and no more than a 0.7% weight lost was achieved at 100 ppm of SM and LM (Figure 1 SW, SM LW and LM). In the LW treatment at 100,000 ppm, 22 and 30 d after the first application, a 28% increase in weight, relative to initial weight, was observed, while with the other concentrations the variation in weight was minimal (Figure 1 LW). The weight variation of the control group was 0.31 to 2.34%. The weight of the weevils 30 d after the first application of SW, SM and LM at all concentrations varied from about -7% to 2.8% (Figure 1 SW, SM and LM). The LM treatment at .1, 1, 10, 1,000, and 10,000 ppm caused a decrease of 0.4, 0.5, 2.7, 7 and 0.3% of initial weight (Figure 1 LM). In contrast, SM at 10,000 decreased initial weight by 0.9% (Figure 1 SM).

3.5.2 Second application. Seven days after the second application of SW extract, insects lost weight at 10, 100 and 1,000 ppm (16, 22, and 17%, respectively) (Figure 2 SW). Fifteen days after the second application of SM, LW and LM at concentrations of 0.1 and 1 ppm, the weevils had lost 1.4 to 9.5% of their initial weight. Twenty-two days after the second application, all of the treatments caused weevils to lose and/or gain weight (Figure 2). The SM treatment at concentrations of 0.1 and 1 ppm caused weight loss of 19 and 17.5% of initial weight (Figure 2 SM). With the treatments of LW and LM at 0.1 and 1 ppm, the weevils had weight losses of 18.5 and 16.8%, respectively (Figure 2 LW and LM). The weight of the control group varied from -6.72 to 0.24% of their initial weight.

3.5.3 Third application. The SW, LW and LM treatments at 7, 15, and 22 d after the third application caused weevil weight losses of no more than 4% and weight gains of up to 4.75% (Figure 3 SW, LW and LM). In the SM treatment, the largest weight loss (-6.25%) occurred 22 d after the third application at 10 ppm. However, at 100,000 ppm, an increase of more than 25% was observed 7 d after the third application, very similar to the increases at 15 and 22 d after this same application (Figure 3 SM). Weight of the control group varied -0.08 to 0.7%.

4. DISCUSSION

The effectiveness of a molecule as an insecticide, whether synthetic or natural, depends on its chemical nature, the amount of active ingredient or ingredients used, the number of applications and the time interval between applications, as well as the area and time of contact with the insect and its size, age and gender (Lagunes-Tejeda and Vázquez-Navarro, 1994). In this study, several areas of agave snout weevils were subjected to different castor bean extracts, concentrations and times of exposure.

Acute application of the extracts in all of the concentrations did not cause severe mortality. A possible explanation is that the extract was not able to break physical barriers to penetrate into the insect and, therefore, did not have a toxic effect, even though acetone was used as a solvent, and organic solvents facilitate deposition and penetration of insecticides into the insect cuticle. Or, perhaps, the molecules evaluated penetrated but did not have a toxic effect on the weevils. Another possible explanation is the time of extract preparation. The aqueous extract of castor bean seeds macerated by 24 h prior to application caused 50% mortality in the chili weevil *Anthonomus eugenii* Cano (Coleoptera: Curculionidae). However, the same extract macerated by 72 h prior use was not active, indicating that this extract must be used 24 h after its preparation or losses its toxicity (Palma and Serrano, 1997). Extraction type can affect insecticide activity. Lagunes-Tejeda (1993) indicated that the extract of castor bean macerated and prepared as an infusion can control the borer *Spodoptera frugiperda* Smith (Lepidoptera: Noctuidae) and the Mexican bean beetle *Epilachna varivestis* Mulsant (Coleoptera: Coccinellidae), while in powder form it is effective against the brown bean weevil *Acanthoscelides obtectus* Say (Coleoptera: Bruchidae).

Collavino et al. (2006) mention that castor bean leaf powder is effective against meal moth larvae *Plodia interpunctella* HBN (Lepidoptera: Phycitinae), while 1% castor bean powder achieved total control of the rice weevil *Sitophilus oryzae* L. (Niber, 1994) and the oil of castor bean seeds at 5,000 and 10,000 ppm caused 40 and 53.7% mortality in adults 192 h after application (Cerna et al., 2010). Commercial castor oil at 5,000 ppm caused 20.87% mortality among adult bean weevil *Zabrotes subfasciatus* (Boh.) (Coleoptera: Curculionidae) 42 d after application (Mushobozy et al., 2009). Insecticide effect of castor bean extracts cannot be attributed to one active compound, but to a mixture of compounds and the proportion in which they are found in the extract. Petroleum ether and ethanolic extracts at 15,000 ppm caused 89 and 60% *S. oryzae* mortality, respectively (Calle et al., 1996), when the petroleum ether was fractionated; the fractions G1 and II at 12,500 and 15,000 ppm kill all the weevils. Ramos-López et al (2010) state that the insecticide and insectistatic activity of methanolic castor-bean-seed extract against *S. frugiperda* may be due to the presence of ricinin. Upasani et al. (2003) report insecticide, ovicide and oviposition dissuasion effects of aqueous castor bean leaf extract on the Chinese bean weevil *Callosobruchus chinensis* L. (Coleoptera: Curculionidae); they identified quercetin as its principal flavonoid. This study on the agave snout weevil, however, did not achieve mortality of the insect with applications of hydroethanolic extracts, even though quercetin was detected in the leaf extracts (LM and LW) evaluated.

It is possible that the metabolites contained in the hydroethanolic castor bean extracts had some effect on the biology and reproduction of the weevil, as has been reported for other curculionids. For example, Tinzaara et al. (2006) report that when a 20% aqueous extract of castor bean leaves was applied, there was an effect on the oviposition of the banana weevil *Cosmopolites sordidus* Germar (Coleoptera: Curculionidae), while Calle et al. (1996) reported that when the fraction G1-2 of the petroleum ether extract was applied at 1000 ppm on rice grains, it caused a 45% reduction in adult emergence of the rice weevil *S. oryzae*. Moreover, these authors report a delayed effect on the reproductive cycle of the insects due to the methyl and ethyl ester compounds of saturated fatty acids.

The extracts applied to a third of the weevils 7, 15 and 22 d after the first application simulated field conditions in which botanical insecticides are applied repeatedly, although the lowest mortality observed indicates a null insecticide effect of the extracts evaluated. Palma and Serrano (1997), however, recommend the use of aqueous extract from castor bean seeds in the field to control the chili weevil *A. eugenii*.

The results of the first application on mortality by gender show that male's casualties were almost the double than those of females. This coincides with the observation of Busvine (1971) that males are more susceptible than females to the application of a toxic substance. Nevertheless, the mortality caused by the treatments of this study was low. Weevils weight loss caused by LM extract could be due to the presence of alkaloids and flavonoids (for example, ricin, ritin and quercetin). Singh and Chauhan (2009) isolated the flavonoids rutin and quercetin from the methanolic extract of *R. communis* leaves; this agrees with our study. Ramos-López et al. (2010) evaluated the effect of ingested ricin oil, ricinin, and hexanic, acetatoethylic and methanolic extracts from 16 to 24,000 ppm on first instar *S. frugiperda* larvae. All treatments with ricinin (560 ppm) and acetatoethylic extract (1600 ppm) from *R. communis* seeds reduced weight of the pupae by 21.6% to 4.9%, respectively; there was an inverse relationship between increase in concentration and weight reduction, a tendency observed in our study with the LM and SM extracts applied on adult weevils.

In the ingestion bioassay, the agave snout weevil was in contact with pieces of agave treated with extracts, simulating field conditions in which the insect looks for food, a place to lay eggs or refuge in the plant. However, no toxic effect on the weevil was obtained. This may be because it did not feed on the treated agave due to a possible repellent effect. Palma and Serrano (1997) mentioned that when leaf discs of chili *Capsicum annum* (Solanaceae) were submerged for 10 seconds in an aqueous extract of castor bean seed (125,000 ppm, maceration 72 h previous) there was a phagorepellant effect on the chili weevil *A. eugenii*, which consumed only 1.4% of the leaf discs. It could also be that the agave snout weevil has the capacity of detoxifying the substances it eats. Studies in this sense have been illustrated by the aliesterases of the cotton boll weevil *Anthonomus grandis* Boheman (Yuan and Chambers, 1998), proteinases of the weevil *Sitophilus zeamais* (Silva et al., 2010), glutation S-transferase of the grain weevil *Sitophilus granarius* L. (Starratt and Bond, 1981) (Coleoptera: Curculionidae). Our study show that the hydroethanolic extracts from leaves and seeds of wild and Mirante variety *R. communis* were not toxic for adult agave snout weevils under our conditions; however, several did affect the insects' weight. It is important to continue this research and, therefore, we recommend assessment of other extracts of *R. communis* (for example, hexanic and methanolic) against adult agave snout weevils and likewise, together with the hydroethanolic extracts, against the larvae in order to have a more precise panorama of their effects.

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TABLE 1. CHEMICAL GROUPS PRESENT IN THE HYDROETHANOLIC, METANOLIC AND HEXANIC EXTRACTS FROM LEAVES AND SEEDS OF WILD AND MIRANTE VARIETY CASTOR BEANS.

Chemical groups	Plant material	Extracts					
		LHet	LMet	LHex	SHet	SMet	SHex
Essential oils	W	√	√	√	√	√	√
	M	√	√	√	√	√	√
Fatty acids	W				√	√	√
	M			√	√	√	√
Terpenes	W						
	M	√	√	√		√	√
Alkaloids	W	√	√				
	M	√	√				
Flavonoids	W	√	√				
	M	√	√				
*Rutin	W	√					
	M	√					
*Quercetin	W	√					
	M	√					
*Quercetrin	W	√					
	M	√					

W= wild castor bean, M= Mirante variety castor bean,

LHet= hydroethanolic extract from leaf, LMet= metanolic extract from leaf, LHex = hexanic extract from leaf, SHet= hydroethanolic extract from seed, SMet= methanolic extract from seed, and SHex= hexanic extract from seed.

*Flavonoids.

TABLE 2. MORTALITY OF FEMALE *S. ACUPUNCTATUS* 168 H AFTER THE FIRST ACUTE APPLICATION OF *R. COMMUNIS* HYDROETHANOLIC EXTRACTS

Concentration (ppm)	Treatments					Total dead females	Mortality (%)
	C	SW	SM	LW	LM		
0	2					2	1.14
10				1		1	0.57
100						1	0.57
1000			1	2		3	1.71
100,000		1				1	0.57
Total	2	1	1	3	0	8	4.57

SW= wild seed, SM= Mirante seed, LW= wild leaf, LM= Mirante leaf, and C= control (N= 175).

TABLE 3. MORTALITY OF *S. ACUPUNCTATUS* MALES 168 H AFTER THE FIRST ACUTE APPLICATION OF *R. COMMUNIS* HYDROETHANOLIC EXTRACTS.

Concentration (ppm)	Treatments					Total dead males	Mortality (%)
	C	SW	SM	LW	LM		
0	4					4	2.28
0.1		1		1		2	1.14
1		1			2	3	1.71
10			1			1	0.57
100			1	1		2	1.14
1000				1		1	0.57
10,000			1	1		2	1.14
1000,000		2		1		3	1.71
Total	4	4	3	5	2	18	10.28

SW= wild seed, SM= Mirante seed, LW= wild leaf, LM= Mirante leaf, and C= control (N=175).

TABLE 4. MORTALITY OF FEMALE *S. ACUPUNCTATUS* 168 H AFTER THE SECOND ACUTE APPLICATION OF *R. COMMUNIS* HYDROETHANOLIC EXTRACTS.

Concentration (ppm)	Treatments					Total dead females	Mortality (%)
	C	SW	SM	LW	LM		
0.1		2				2	2.27
100					1	1	1.13
10,000			1	1		2	2.27
Total	0	2	1	1	1	5	5.68

SW= wild seed, SM= Mirante seed, LW= wild leaf, LM= Mirante leaf and C= control (N=88).

TABLE 5. MORTALITY OF MALE *S. ACUPUNCTATUS* 168 H AFTER THE SECOND ACUTE APPLICATION OF HYDROETHANOLIC *R. COMMUNIS* EXTRACTS.

Concentration (ppm)	Treatments					Total dead males	Mortality (%)
	C	SW	SM	LW	LM		
0	1					1	1.14
100		1				1	1.14
Total	1	1	0	0	0	5	2.29

SW= wild seed, SM= Mirante seed, LW=wild leaf, LM= Mirante leaf, and C= control (N=87).

TABLE 6. MORTALITY OF FEMALE *S. ACUPUNCTATUS* 168 H AFTER THE THIRD ACUTE APPLICATION OF HYDROETHANOLIC EXTRACTS OF *R. COMMUNIS*.

Concentration (ppm)	Treatments					Total dead females	Mortality (%)
	C	SW	SM	LW	LM		
0.1			1			1	2.0
10					1	1	2.0
Total	0	0	1	0	1	2	4.0

SW= wild seed, SM= Mirante seed, LW= wild leaf, LM= Mirante leaf, and C= control (N=50).

TABLE 7. ACCUMULATED MORTALITY OF FEMALE *S. ACUPUNCTATUS* OVER 7 D OF INGESTING HYDROETHANOLIC *R. COMMUNIS* EXTRACTS AT 10,000 PPM APPLIED ON TEQUILA AGAVE.

Days	Treatments					Total dead females	Mortality (%)
	C	SW	SM	LW	LM		
2			1			1	0.50
5		1			1	2	1.00
7	2					2	1.00
Total	2	1	1	0	1	5	2.50

SW= wild seed, SM= Mirante seed, LW= wild leaf, LM= Mirante leaf, and C= control (N=200).

TABLE 8. MORTALITY OF MALE *S. ACUPUNCTATUS* ACCUMULATED OVER 7 DAYS OF INGESTING HYDROETHANOLIC *R. COMMUNIS* EXTRACTS AT 10,000 PPM APPLIED ON TEQUILA AGAVE.

Days	Treatments					Total dead males	Mortality (%)
	C	SW	SM	LW	LM		
1			1	1		2	1.00
3		1	1		2	4	2.00
7			1	1		2	1.00
Total	0	1	3	2	2	8	4.00

SW= wild seed, SM= Mirante seed, LW= wild leaf, LM= Mirante leaf, and C= control (N=200).

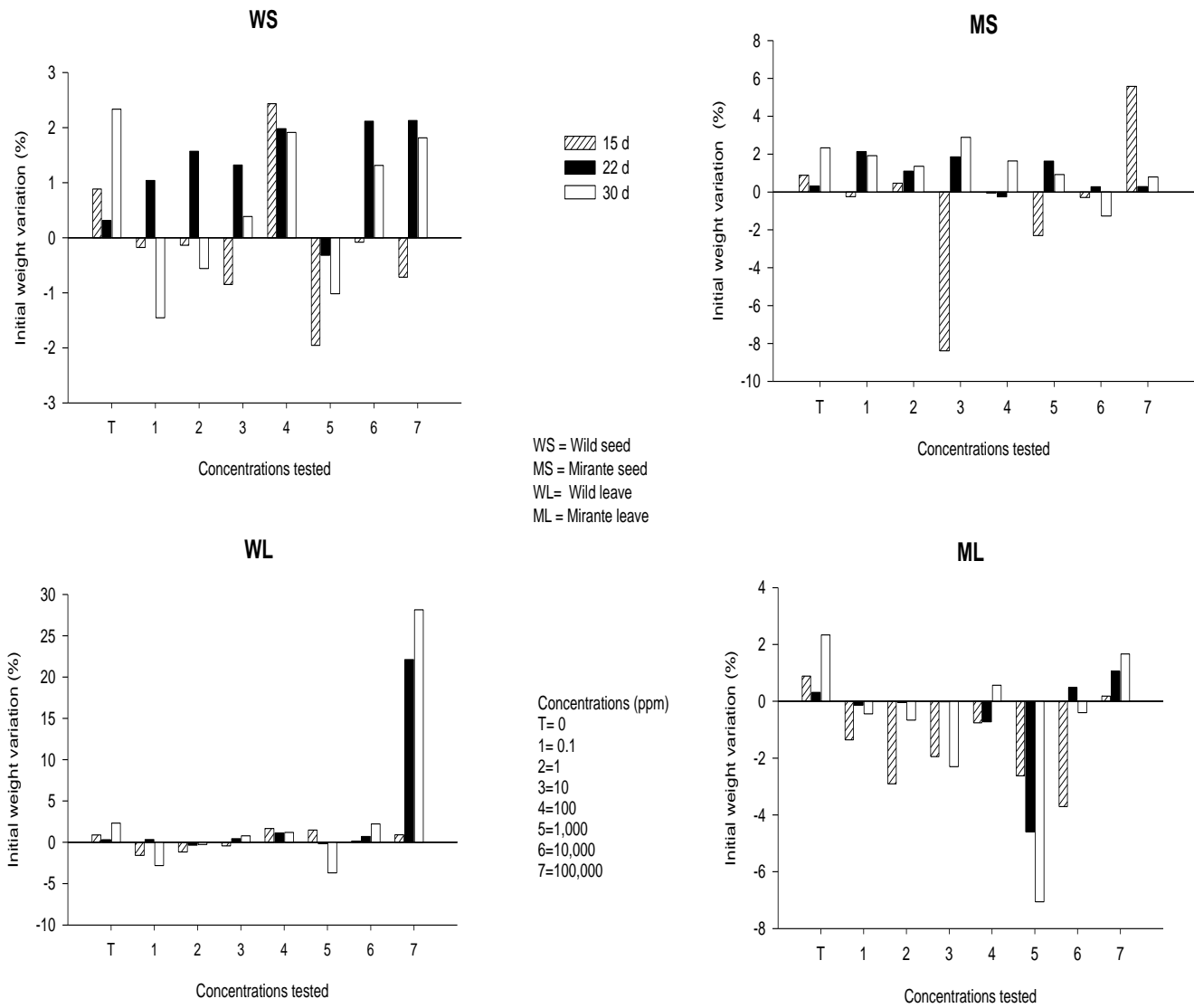


Figure 1. *S. acunpuntatus* female and male initial weight variation (%) 15, 22 and 30 d after the first acute *R. communis* hydroethanolic extracts application.

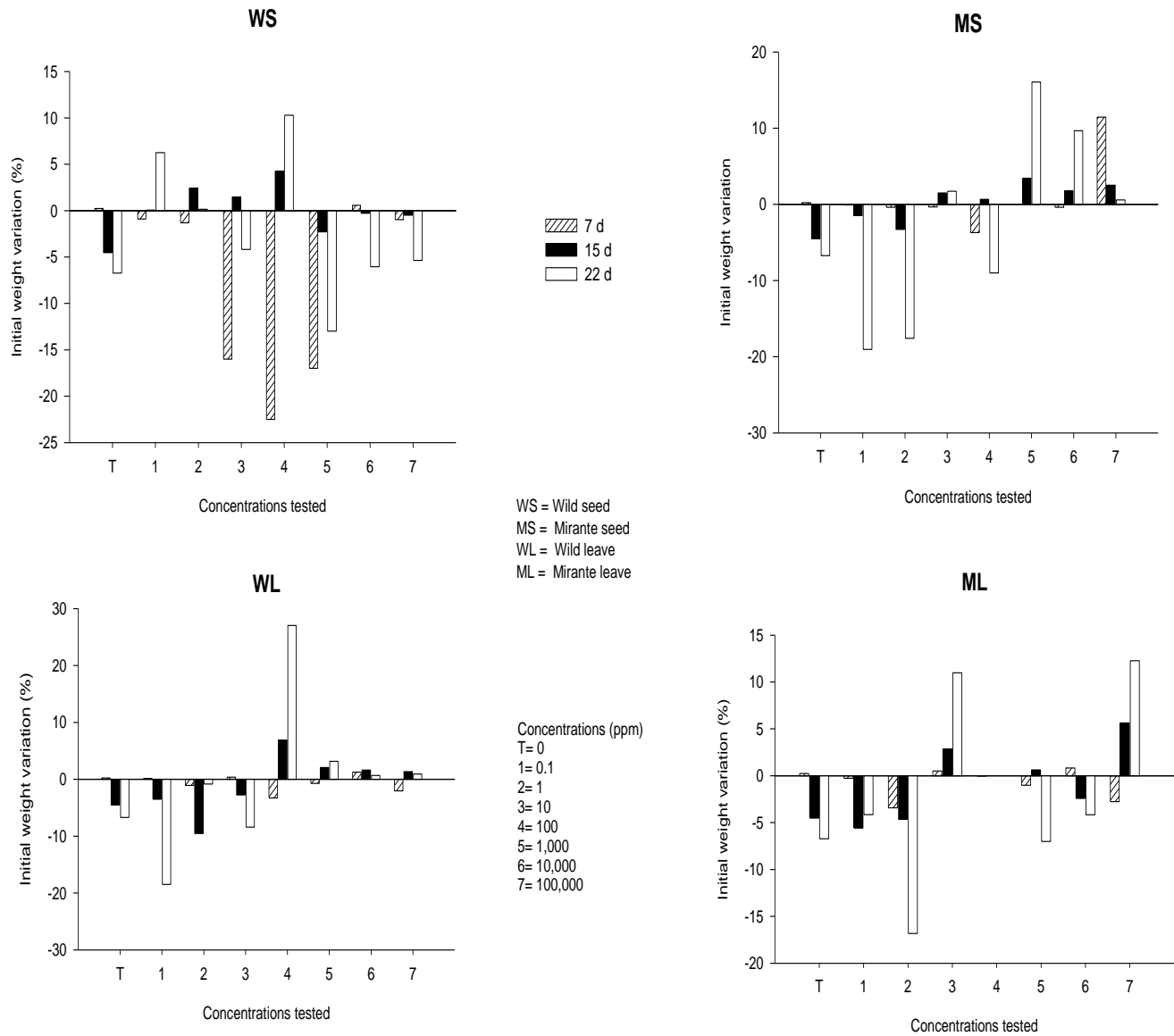


Figure 2. *S. acupunctatus* female and male weight variation (%) 7, 15 and 22 d after the second acute *R. communis* hydroethanolic extracts application

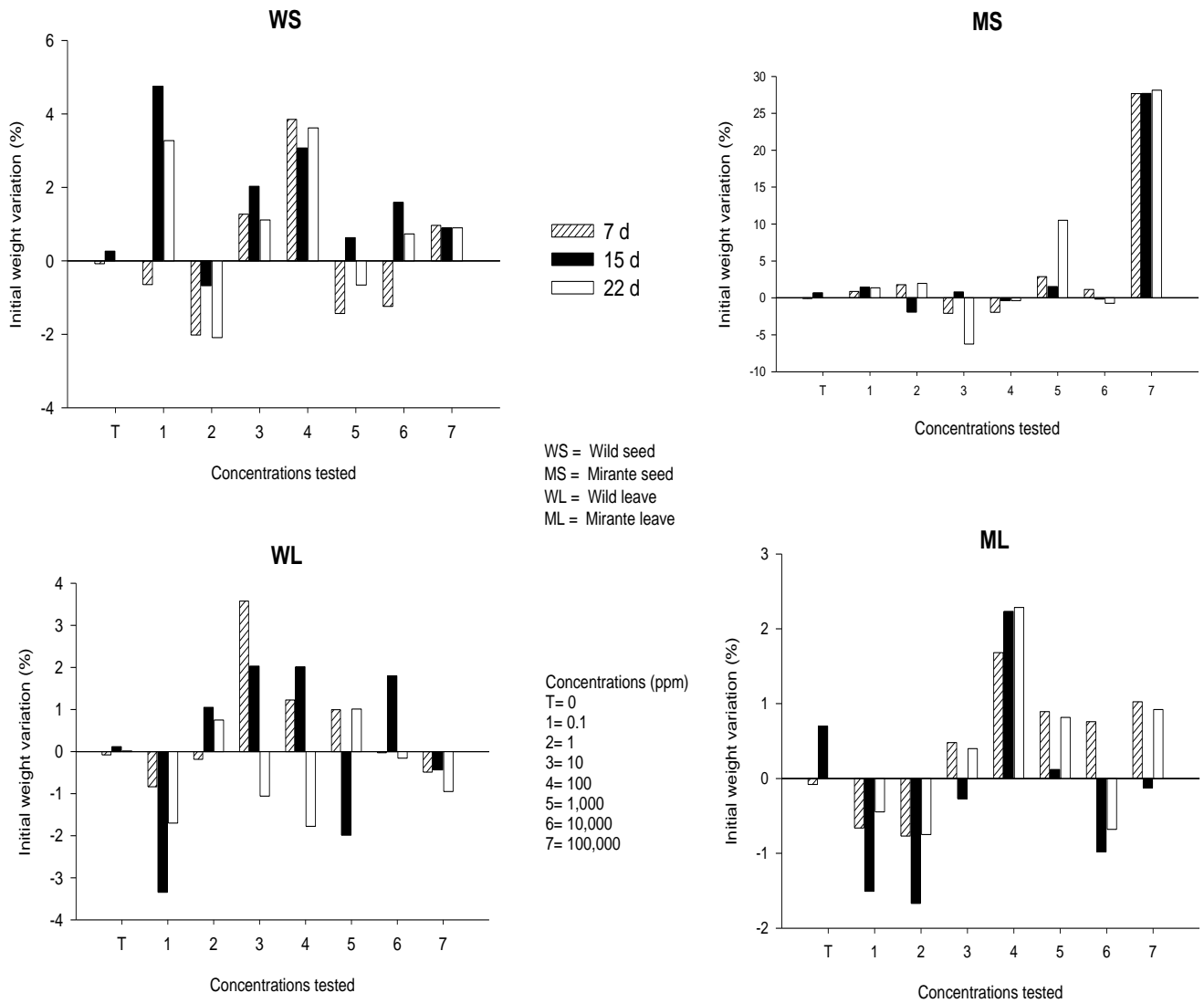


Figure 3. *S. acupunctatus* female and male initial weight variation (%) 7, 15 and 22 d after the third acute *R. communis* hydroethanolic extracts application