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1 **TITLE: Selection of flowering plants to enhance the biological control of *Tuta absoluta* using**
2 **parasitoids**

3

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9 **Abstract**

10 The tomato moth *Tuta absoluta* is an important pest of tomato crops. To enhance biological control
11 services, our study aims to ascertain the effect of potential insectary plants (*Achillea millefolium*,
12 *Calendula officinalis*, *Fagopyrum esculentum*, *Lobularia maritima*, *Ononis natrix*, *Sinapis alba*, and
13 *Tagetes patula*) as nutrient-providers to facilitate the installation of *T. absoluta* parasitoids
14 (*Necremnus tutae*, *Stenomesus* nr. *japonicus*, and *Bracon* nr. *nigricans*) without encouraging the
15 pest. Our results shown that different flowers provide different benefits in terms of survival and egg
16 load for different parasitoid species, and that the benefit of these flowers for *T. absoluta* females was
17 low. Being *N. tutae* the most abundant and widespread parasitoid, selection of flowers might target
18 this parasitoid. However, the provision of a flower mixture may be more advantageous than selecting
19 only one species because they may contribute to enhance a broader guild of parasitoid wasps.

20

21 **Keywords:** Gelechiidae; Eulophidae; Braconidae; Conservation biological control; Ecological
22 infrastructures; Plant foods

23

25 1. Introduction

26 The tomato moth *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) was first detected in Spain in
27 2006 (Urbaneja et al. 2007) and from there, it spread quickly both across Europe, the Mediterranean
28 area, Asia and Africa causing major damage to tomato crops (Campos et al. 2017; Desneux et al.
29 2010, 2011; Urbaneja et al. 2013). Biological control of pests in tomato both in greenhouse and
30 outdoor crops is based mainly on the use of the predatory mirid bugs *Macrolophus pygmaeus*
31 (Rambur) and *Nesidiocoris tenuis* Reuter (Hemiptera: Miridae) (Albajes et al. 2003; Gabarra et al.
32 2008; Jaworski et al. 2015; Shaltiel-Harpaz et al. 2016; Urbaneja et al. 2012). These predators are
33 effective controlling *T. absoluta* by feeding on the eggs of the moth, but they have a poorer
34 performance feeding on larvae (Arnó et al. 2009; Urbaneja et al. 2009). To complement the activity
35 of these predators larval parasitoids may be of great help. In the Mediterranean area, numerous species
36 of parasitoids of the *T. absoluta* larvae have been found, belonging mostly to the families Eulophidae,
37 Braconidae, and Ichneumonidae. Among the most abundant are *Necremnus* sp. nr. *artynes* Walker,
38 *Stenomesus* sp. nr. *japonicus* Ashmed (Hymenoptera: Eulophidae), and *Bracon* sp. nr. *nigricans*
39 Szépligeti (Hymenoptera: Braconidae) (Biondi et al. 2013a; Ferracini et al. 2012; Gabarra et al. 2014;
40 Urbaneja et al. 2012; Zappalà et al. 2012, 2013). Using an integrative taxonomy approach, Gebiola
41 et al. (2015) revised the different species of *Necremnus* found parasitizing *T. absoluta* and discovered
42 four new species, one of them being *Necremnus tutae* (Ribes & Bernardo). According to these
43 authors, *N. tutae* is the most abundant species in the field, with the largest geographical range and
44 highest parasitism on *T. absoluta*. These authors indicate that many studies conducted in Europe with
45 *N. artynes* or *N. sp. nr. artynes* should actually refer to *N. tutae*.

46 In general, parasitoids play an important role in biological pest control, due to their ability to
47 regulate the populations of many phytophagous insects. Many of these parasitoids reach their
48 maximum biological potential when they have an adequate food supply. Sugar rich diets have a

49 positive effect on longevity and/or reproduction of parasitoids (Benelli et al. 2017; Heimpel and
50 Jervis, 2005; Jervis et al. 2008) and, therefore, availability of sugar rich sources, such as nectar,
51 increase parasitoid density and enhance the biological control service provided by parasitoids (Tena
52 et al. 2015). In nature, parasitoids can obtain carbohydrates from floral and extrafloral nectar,
53 honeydew, fruits (Tena et al. 2016; Wäckers, 2005; Wäckers et al. 2008) and from host feeding (Giron
54 et al. 2002). In addition of being a sugar-rich diet, nectar contains minor quantities of amino acids,
55 organic acids, lipids, proteins and inorganic ions (Nicolson and Thornburg, 2007) that may be
56 important for the ovigenesis (Jervis et al. 2008). Regarding *T. absoluta* parasitoids, Balzan and
57 Wäckers (2013) reported an increase in the longevity of *N. artynes* adults when feeding on floral
58 nectar of some flowers and the pest was not available. Being synovigenic *N. tutae*, *S. nr. japonicus*
59 and *B. nr. nigricans* need additional nutrients to produce eggs. The oogenesis is usually associated
60 with a protein-rich diet that these parasitoids mostly obtain by feeding on *T. absoluta* larvae (Balzan
61 and Wäckers, 2013; Biondi et al. 2013b; Calvo et al. 2013; Chailleux et al. 2014).

62 However, agroecosystems are today dominated by monocultures where floral resources are
63 scarce which can negatively affect biological control (Heimpel and Jervis, 2005; Landis et al. 2000).
64 Therefore, there is a growing interest in the reintroduction of floral resources in crops as a strategy to
65 provide the necessary food, such as nectar, for natural enemies (Geneau et al. 2012; Parolin et al.
66 2012; Winkler et al. 2009a). To introduce these floral resources, certain factors must be taken into
67 consideration, for example, the abundance and distribution of flowers, their nutritional value, and the
68 degree of attraction and accessibility of nectar (Wäckers, 2004). The positive effect of flowers on the
69 proliferation of pests must also be considered, and the overall effect will depend on the impact of
70 floral resources on the biological parameters of both the parasitoid and the pest. In the short-term, the
71 herbivores may benefit from the presence of flowers, but, in the long-term, parasitoid populations can
72 increase sufficiently to prevent an increase in the pest population (Wäckers et al. 2007).

73 To enhance the biological control of *T. absoluta*, the present study aims to select potential
74 insectary plants that may provide nutrients to facilitate the early installation of parasitoids without
75 encouraging the pest. To do that, we have determined the effect of several floral resources on the
76 survival and egg load of the *T. absoluta* parasitoids *N. tutae*, *S. nr. japonicus*, and *B. nr. nigricans*.
77 Likewise, the effect of the floral resources on the pest survival was also evaluated.

78

79 **2. Material and Methods**

80 **2.1 Insects and plant material**

81 All experiments and insect rearings were conducted at IRTA facilities (Cabrils, Barcelona, Spain)
82 under controlled conditions of $25 \pm 1^\circ\text{C}$, $70 \pm 10\%$ RH and 16:8 h (L:D) photoperiod. Insect colonies
83 were initiated in 2011 using individuals collected from tomato crops infested with *T. absoluta* near
84 Barcelona (Spain). *Tuta absoluta* was reared on caged tomato plants (var. Marmande). *Necremnus*
85 *tutae* (identified by Ribes and Bernardo) (Gebiola et al. 2015), *S. nr. japonicus*, and *B. nr. nigricans*
86 individuals were reared on caged tomato plants infested with *T. absoluta* similarly to Chailleux et al.
87 (2014). To obtain individuals for the experiments, leaflets with parasitized larvae were kept in an
88 aerated cage with a paper impregnated with honey until the parasitoid and moth adults emerged and
89 were three to four days old, to ensure mating (Biondi et al. 2013b; Chailleux et al. 2014).

90 The tested plant species were: *Achillea millefolium* L., *Calendula officinalis* L., and *Tagetes*
91 *patula* L. belonging to the family Asteraceae; *Lobularia maritima* L. and *Sinapis alba* L. belonging
92 to the family Brassicaceae; *Fagopyrum esculentum* Moench belonging to the family Polygonaceae,
93 and *Ononis natrix* L. belonging to the family Fabaceae. *Achillea millefolium*, *F. esculentum*, *L.*
94 *maritima*, *S. alba*, and *T. patula* were selected because they have potential to enhance parasitoid
95 activity (Bosch et al. 1997; Dib et al. 2012; Geneau et al. 2012; Rahat et al. 2005, Vattala et al. 2006;
96 Wäckers, 2004; Winkler et al. 2009b), even in Mediterranean agroecosystems (Alomar et al. 2008;

97 Arnó et al. 2012). *Calendula officinalis* and *O. natrix* were proposed by Alomar et al. (2006) to be
98 used as insectary plants to promote the presence of the polyphagous predatory mirid bugs close to
99 tomato crops and, therefore, to contribute to the biological control of several pests. These plants, and
100 especially *C. officinalis*, are currently used in tomato commercial farms to keep during crop-free
101 periods (RG and JA, unpublished). All the flowers used in the assays were obtained from plants
102 cultivated in a greenhouse in pots with potting compost until blooming. Seedlings of *A. millefolium*,
103 *C. officinalis*, *L. maritima* and *T. patula* were bought from a local producer and young plants of *O.*
104 *natrix* were collected in non-cultivated environments in our area. *Fagopyrum esculentum* and *S. alba*
105 plants were grown from commercial seeds. Tomato plants (var. Marmande) were grown from seeds
106 in trays with multiplication substrate and kept in a greenhouse until use.

107

108 **2.2 Effect of the presence of flowers on survival and egg load of parasitoids**

109 **2.2.1 Survival**

110 As an indicator of the effect of flower resources on the life-span of different parasitoid species, their
111 survival was measured during 15 days. Mated and honey-fed females and males (3-4 days old) of
112 *S. nr. japonicus*, *N. tutae*, and *B. nr. nigricans* were kept individually in plastic cups (11 cm high x 9
113 cm diameter) with top ventilation and daily mortality was recorded. The duration of the experiment
114 was chosen since data on longevity of the three parasitoid species ranged from 15 to 62 days when
115 hosts were available (Balzan and Wäckers, 2013; Biondi et al. 2013b; Calvo et al. 2013; Chailleux et
116 al. 2014). Each plastic cup had a small hole in the base to insert a stem with a flower or an
117 inflorescence to assure nectar supply (25-40 open flowers). The plastic cup was put on a smaller one
118 (10 cm high x 7.7 cm diameter) containing water. The stem was left long enough to be submerged in
119 the water, and the hole around the stem sealed with filler to prevent the escape of parasitoids. The
120 flower resources were the inflorescences of *A. millefolium*, *F. esculentum*, *L. maritima*, *O. natrix*, and

121 *S. alba*, and just one flower of *C. officinalis* and *T. patula*. Two controls without flowers were
122 included: one with commercial multi floral honey spread on a piece of filter paper (12 x 2 cm) and
123 the other with only water, provided in a plastic tube plugged with a cotton ball. The flowers, honey,
124 and water were renewed twice a week, since Wade and Wratten (2007) show that, in several plant
125 species including *L. maritima* and *F. esculentum*, parasitoid longevity was similar when fed on
126 excised flowers that were changed every 2 to 5 days than when fed on intact flowers. Fifteen
127 individuals were evaluated by gender and species for each food treatment.

128

129 **2.2.2 Egg load**

130 Egg load is considered a parameter related to the reproductive potential of parasitoids (Benelli et al.
131 2017). To measure the effect of several flowers on the egg load of *T. absoluta* parasitoids, the same
132 experimental system and treatments as described in the previous section were used. In each
133 experimental unit, 3-4 days-old honey-fed mated females were introduced for 48 hours. After this
134 time, the surviving females were frozen at -20°C until dissection. To dissect them, the females were
135 placed on a microscope slide and the thorax separated from the abdomen with a scalpel. The anal end
136 of the abdomen was cut and the individual fully covered with a drop of Ringer's solution. The ovaries
137 were observed under a stereomicroscope and the number of chorionated oocytes recorded. Females
138 in cages with honey and with water were used as controls. The egg load of 15 females was measured
139 for each treatment.

140

141 **2.3 Effect of presence of flowers on *Tuta absoluta* survival**

142 The experimental system and treatments described for its parasitoids was also used to measure the
143 effect of flowers on the survival of males and females of *T. absoluta*. The treatments evaluated in this

144 case were the flowers of the seven plant species, honey, a solution of honey and water (10%), and
145 water (control). A total of 15 individuals of *T. absoluta* were evaluated by gender and for each
146 treatment.

147

148 **2.4 Data analysis**

149 Kaplan–Meier estimates were used to determine the survival functions of males and females of
150 *T. absoluta* and three of its parasitoid species when provided with different foods. Log-rank tests were
151 used to compare among all different survival curves and a complete pairwise analysis was performed.
152 The Bonferroni correction was used to find statistical differences for each pair of treatments. Analyses
153 were done using SigmaPlot 13. Egg load of parasitoids was compared using a generalized linear
154 model (GLM), based on a Poisson distribution using a log link function. When statistically significant
155 differences appeared in the GLM, means were separated using Tukey's post-hoc test ($P < 0.05$) for
156 multiple comparisons. These statistical analyses were performed using R software.

157

158 **3. Results**

159 **3.1 Effect of the presence of flowers on survival and egg load of parasitoids**

160 **3.1.1 Survival**

161 Significant differences in survival curves were observed for *N. tutae* (Fig. 1), *S.nr. japonicus* (Fig. 2)
162 and *B. nr. nigricans* (Fig. 3) females ($\chi^2_8=64.74$, $P < 0.0001$; $\chi^2_8=36.45$, $P < 0.0001$; $\chi^2_8=67.49$, $P <$
163 0.0001 ; respectively) and males ($\chi^2_8=53.34$, $P < 0.0001$; $\chi^2_8=40.86$, $P < 0.0001$; $\chi^2_8=78.99$, $P < 0.0001$,
164 respectively). The χ^2 and P values of each pairwise analysis between survival curves are shown in
165 Table 1. Out of the 90 unfed (provided with only water) individuals studied belonging to the three
166 parasitoid species, only one female of *B. nr. nigricans* survived to the end of the experiment. Provision

167 of a sugar-rich diet such as honey significantly increased the survival of females and males of *N. tutae*
168 and *B. nr. nigricans* compared to unfed individuals, but not of *S. nr. japonicus*. Flowers of *F.*
169 *esculentum* significantly improved the survival of *N. tutae* females and *B. nr. nigricans* females and
170 males. Individuals of both sexes of *N. tutae* fed with *L. maritima* flowers survived significantly longer
171 than the unfed ones and the same effect was observed for *B. nr. nigricans* males. None of the other
172 flowers had a significant positive effect on the survival of these two parasitoids. Regarding
173 *S. nr. japonicus*, none of the tested flowers significantly increased its survival compared to individuals
174 that were given access to only water, although survival in *L. maritima* at the end of the experiment
175 was 47%.

176

177 **3.1.2 Egg load**

178 The presence of some of the flowers increased egg load in females of the three species of *T. absoluta*
179 parasitoids even when they fed for only two days on these resources and, although previously
180 nourished with a sugar rich diet such as honey. *Necremnus tutae* significantly increased the egg load
181 when they fed honey, *F. esculentum*, *L. maritima*, and *O. natrix* compared to unfed females ($\chi^2_8 =$
182 343.92; $P < 0.001$; Fig. 4). *Stenomesus nr. japonicus* females had significantly higher egg load in
183 treatments with *S. alba* and *F. esculentum* than in the treatments with water and honey and other
184 species of tested flowers ($\chi^2_8 = 189.80$; $P < 0.001$; Fig. 4). Finally, females of *B. nr. nigricans* had
185 significantly a higher egg load when they fed on *O. natrix* than on the rest of the treatments ($\chi^2_8 =$
186 211.62; $P < 0.00$; Fig. 4). The availability of *T. patula*, *L. maritima* or honey also significantly
187 increased the egg load of females compared to those who only had water. *Achillea millefolium* and
188 *C. officinalis* did not increase the egg load of any parasitoid species.

189

190 **3.2 Effect of presence of flowers on *Tuta absoluta* survival**

191 Significant differences in survival curves of females and males of *T. absoluta* fed with different food
192 resources were observed ($\chi^2_9=72.83$, $P < 0.0001$ and $\chi^2_9=61.86$, $P < 0.0001$, respectively) (Fig. 5).
193 None of the unfed males and only one unfed female survived to the end of the experiment. As can be
194 observed in Fig.5 and Table 2, provision of a sugar-rich diet such as a honey solution significantly
195 increased the survival of females and males compared to that of unfed individuals. None of the tested
196 flowers significantly improved the survival of females of *T. absoluta* when compared to that of unfed
197 ones. However, five flower species (*A. millefolium*, *C. officinalis*, *F. esculentum*, *L. maritima* and *O.*
198 *natrix*) significantly increased survival of males.

199

200 **4. Discussion**

201 The results obtained in our experiments showed that availability of some flowers had a relevant
202 influence on the survival, measured up to 15 days, and the egg load of the *T. absoluta* parasitoids *N.*
203 *tutae*, *S. nr. japonicus*, and *B. nr. nigricans*. Out of the seven species tested, flowers of *F. esculentum*
204 and *L. maritima* were the ones that most benefit the survival of both males and females of the three
205 *T. absoluta* parasitoids. Survival increases associated with *F. esculentum* and *L. maritima* obtained
206 in this work agree with those obtained by Balzan and Wäckers (2013) for *N. artynes* and with similar
207 studies done with other braconid species (Berndt and Wratten, 2005; Hopkinson et al. 2013;
208 Johanowicz and Mitchell, 2000; Nafziger Jr. and Fadamiro, 2011; Vattala et al. 2006; Winkler et al.
209 2009a).

210 Although female parasitoids fed on honey for 3-4 days and were provided with distinct flower
211 resources for only two days, five out of the seven tested flower species increased the egg load of *T.*
212 *absoluta* parasitoids. Presence of *F. esculentum* flowers increased the egg load of *N. tutae* and *S. nr.*
213 *japonicus*, and *L. maritima* flowers had the same effect on *N. tutae* and *B. nr. nigricans*. Other studies
214 have reported a positive effect of *F. esculentum* and *L. maritima* on the egg load of several braconid
215 wasps (Araj et al. 2008; Geneau et al. 2012; Winkler et al. 2009a; Witting-Bissinger et al. 2008).
216 However, Balzan and Wäckers (2013) reported that the egg load of females of *N. artynes* was similar

217 between females fed on a sucrose solution and young unfed females, what according to the authors,
218 would indicate that this carbohydrate do not contribute to the fertility of this parasitoid. The
219 differences between our results and those of Balzan and Wäckers (2013) may possibly be due to the
220 more complex composition of honey and nectar (Nicolson and Thornburg, 2007) that enhance
221 ovigenesis (Jervis et al. 2008).

222 The availability of *O. natrix* increased the number of mature oocytes in *N. tutae* and *B. nr.*
223 *nigricans*, however, it had no effect on the survival of any of the *T. absoluta* parasitoids included in
224 the study, which suggested that they did not profit from nectar. In fact, it is known that Fabaceae have
225 a very restrictive corolla for hymenoptera (Winkler et al. 2009a), and therefore the increase in egg
226 load registered in our studies would suggest that parasitoids may have consumed a plant resource
227 other than nectar. *Ononis natrix* has abundant glandular trichomes whose exudates cover the plant
228 (Wollenweber and Dorr, 2003), which in some cases can be used as nutrients by the parasitoids. For
229 example, Olson and Nechols (1995) demonstrated that trichome exudates of *Cucurbita pepo* L.
230 contain monosaccharides and some proteins that increase longevity and fecundity of the parasitoid
231 *Gryon pennsylvanicum* (Ashmed) (Hymenoptera: Scelionidae).

232 The effect of *S. alba* nectar on *S. nr. japonicus* egg load was the highest recorded in the whole
233 experiment including three parasitoid and seven flower species, and it quadrupled egg load of unfed
234 females of this species. Possibly, its nectar composition is especially suitable to support egg
235 maturation in this insect. The effect of *S. alba* nectar on parasitoid fitness has not been previously
236 reported in existing literature, although in field surveys, natural parasitism of beetles increased in
237 areas close to margins of this plant species (Manojlovic et al. 2000), suggesting that hymenoptera
238 parasitoids may use these flowers as food resources.

239 *Tagetes patula* flowers increased the egg load of *B. nr. nigricans*, respectively. This agrees
240 with the observations made by Jervis et al. (1993) who reported that braconids can exploit the flowers
241 with narrow and tubular corollas with nectaries at the base, such as heads of Asteraceae the family to
242 which *T. patula* belong.

243 The flowers within and next to fields that improve parasitoid fitness may also improve pest
244 fitness (Wäckers et al. 2007). In our experiments, none of the floral resources provided seemed to be
245 profitable for females of *T. absoluta* but several of them increased male longevity. This may be
246 advantageous for the searching capacity of males since females are polyandrous and mate frequently
247 (Lee et al. 2014). Balzan and Wäckers (2013) also observed that *T. absoluta* was able to utilize nectar
248 resources from flowers, same as other species such as *Phthorimaea operculella* (Zeller) (Lepidoptera:
249 Gelechiidae), *Pieris rapae* L. (Lepidoptera: Pieridae) and *Plutella xylostella* L. (Lepidoptera:
250 Plutellidae) (Baggen and Gurr, 1998; Winkler et al. 2009a). However, the overall benefit of the nearby
251 insectary plants to the fields in terms of biological pest control depends on the balance between the
252 advantages these plants report to herbivores and to their natural enemies (Wäckers et al. 2007;
253 Winkler et al. 2009b, 2010). According to the results of our experiments, the benefits that the tested
254 flowering plants represent for *T. absoluta* females were very similar among the different species and
255 were in general low. With this premise, species that are more favorable to enhance the activity of
256 natural enemies should be chosen as insectary plants. None of the tested flowers increased longevity
257 and egg load of all the three parasitoid species. *Fagopyrum esculentum* and *L. maritima* have shown
258 to be the most beneficial in terms of survival and reproduction for all three parasitoid species and
259 especially for *N. tutae* and *B. nr. nigricans*. Both *F. esculentum* and *L. maritima* are in bloom during
260 summer (Halbrecq et al. 2005; Picó and Retana, 2001) when *T. absoluta* is a serious problem in
261 tomato crops. In addition, *O. natrix*, *S. alba* and *T. patula* have shown to increase egg load and,
262 therefore, they can be useful to enhance reproduction of the parasitoids. *Ononis natrix* it is also very
263 valuable to promote the presence of *M. pygmaeus* (Alomar et al. 2006), a very efficient predator of
264 *T. absoluta* eggs (Arnó et al. 2009; Urbaneja et al. 2009). Therefore, the presence of this plant species
265 close to tomato plants may complement the range of *T. absoluta* natural enemies and enhance the
266 biological control of this pest.

267 Although many larval parasitoids of *T. absoluta* have been identified in the Mediterranean,
268 *N. tutae* is the most widespread and abundant species (Ferracini et al. 2012; Gabarra et al. 2014;

269 Gebiola et al. 2015; Zappalà et al. 2013). In fact, in Spain, natural populations of this parasitoid
270 currently contribute to control *T. absoluta* in greenhouse tomatoes (van der Blom et al. 2016) and in
271 outdoor tomatoes (RG and JA, unpublished) and, in Tunisia, apparent parasitism rates reach up to
272 25.5% (Abbes et al. 2014). Therefore, insectary plants that enhance *N. tutae* fitness have to be selected
273 to improve the biological control of *T. absoluta*. However, as shown by our results, different flowers
274 provided different benefits for different parasitoid species. Consequently, the provision of a flower
275 mixture may be more advantageous than selecting only one species because they may contribute to
276 enhance a broader guild of parasitoid wasps. Further field trials are required to determine what will
277 be the overall effect of these flowering margins in the improvement of biological control of this pest.
278

279

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288

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- 457

458 **Figure 1.** Kaplan – Meier estimates of survivorship up to 15 days of 3-4 days old females and
459 males of *N. tutae* when feeding on different flowers, honey and only water. Different letters in the
460 legend indicate significant differences among treatments after pairwise comparison of the survival
461 curves (Bonferroni correction was used and a value of $P < 0.05/36 = 0.0014$ was considered
462 statistically significant).

463 **Figure 2.** Kaplan – Meier estimates of survivorship up to 15 days of 3-4 days old females and males
464 of *S. nr. japonicus* when feeding on different flowers, honey and only water. Different letters in the
465 legend indicate significant differences among treatments after pairwise comparison of the survival
466 curves (Bonferroni correction was used and a value of $P < 0.05/36 = 0.0014$ was considered
467 statistically significant).

468 **Figure 3.** Kaplan – Meier estimates of survivorship up to 15 days of 3-4 days old females and males
469 of *B. nr. nigricans* when feeding on different flowers, honey and only water. Different letters in the
470 legend indicate significant differences among treatments after pairwise comparison of the survival
471 curves (Bonferroni correction was used and a value of $P < 0.05/36 = 0.0014$ was considered
472 statistically significant).

473 **Figure 4.** Egg load or mean number of mature oocytes per female (\pm standard error) of *S. nr.*
474 *japonicus*, *N. tutae*, *B. nr. nigricans* after feeding on different food sources. Bars with different letters
475 show significant differences between treatments ($P < 0.05$, Tukey's test).

476 **Figure 5.** Kaplan – Meier estimates of survivorship up to 15 days of 3-4 days old females and males
477 of *T. absoluta* when feeding on different flowers, honey and only water. Different letters in the legend
478 indicate significant differences among treatments after pairwise comparison of the survival curves
479 (Bonferroni correction was used and a value of $P < 0.05/45 = 0.0011$ was considered statistically
480 significant).

481

482

483 **Table 1.** Chi-square (χ^2) and P values obtained in the pairwise comparisons of the survival curves
484 using log-rank tests for *N. tuta*, *S. nr. japonicus* and *B. nr. nigricans*. Bonferroni correction was used
485 to weigh the multiple comparisons done ($P < 0.05/36 = 0.0014$ was considered statistically
486 significant).

487 **Table 2.** Chi-square (χ^2) and P values obtained in the pairwise comparisons of the survival curves
488 using log-rank tests for *T. absoluta*. Bonferroni correction was used to weigh the multiple
489 comparisons done ($P < 0.05/45 = 0.0011$ was considered statistically significant).

490

Figure 1

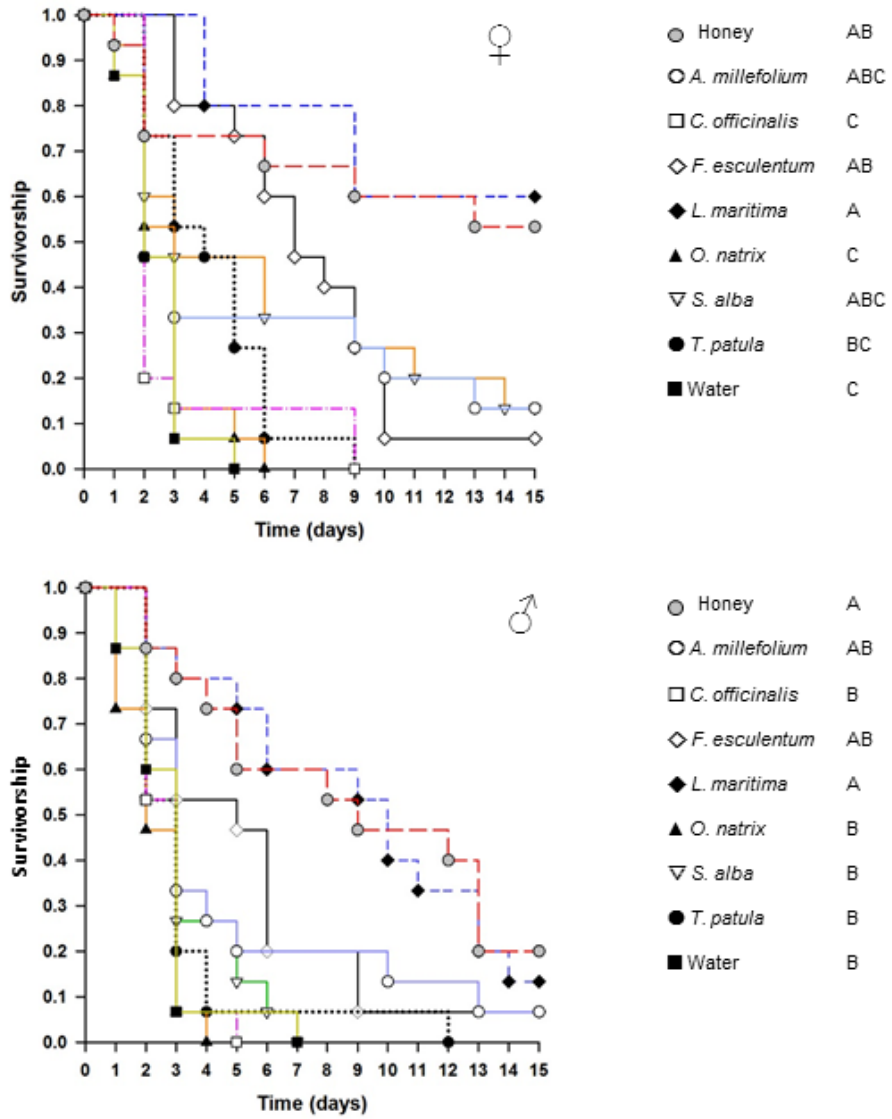


Figure 2.

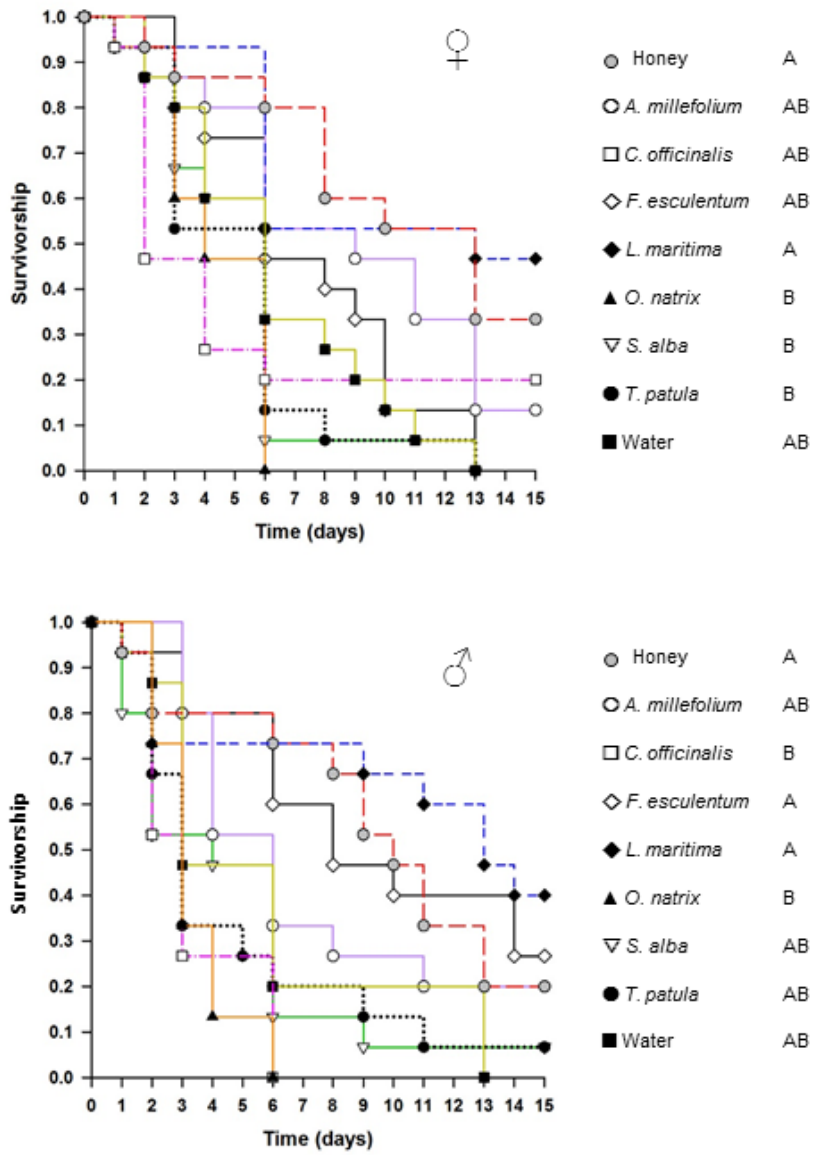


Figure 3.

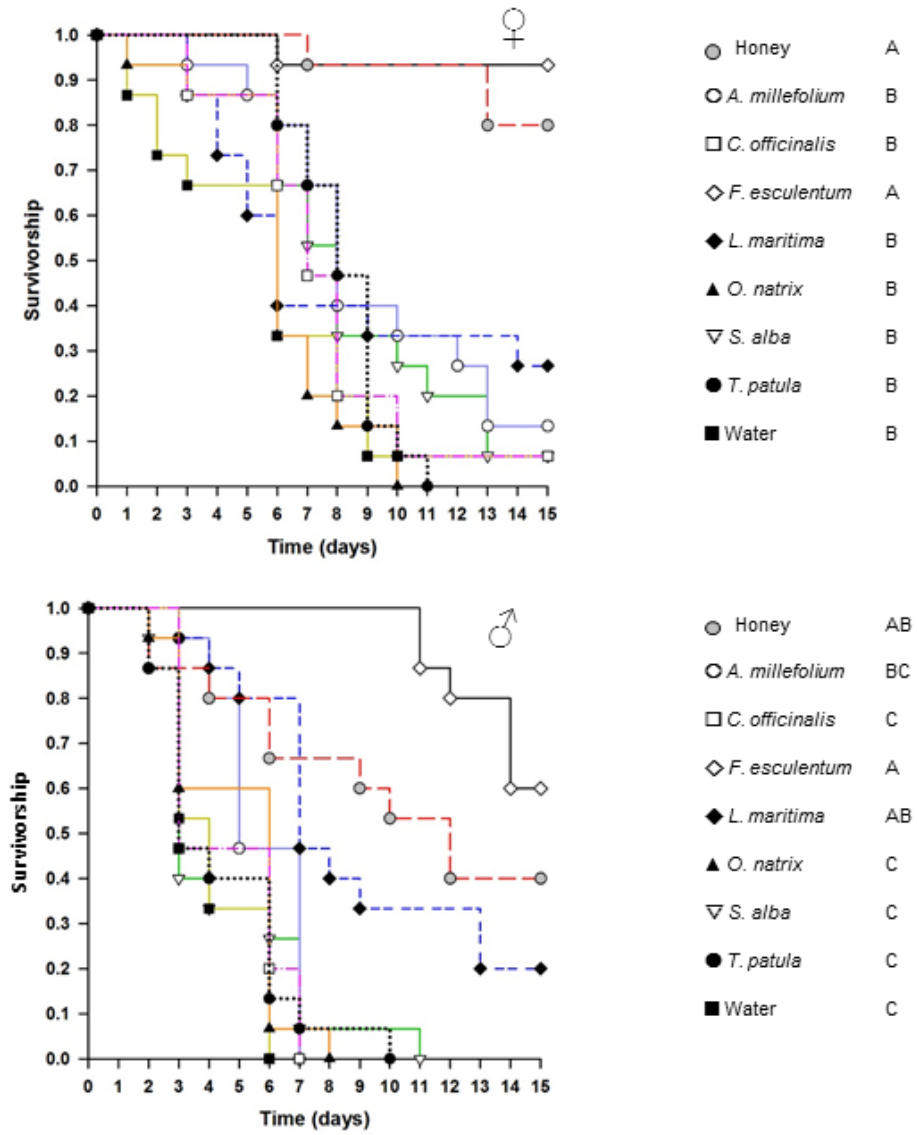


Figure 4.

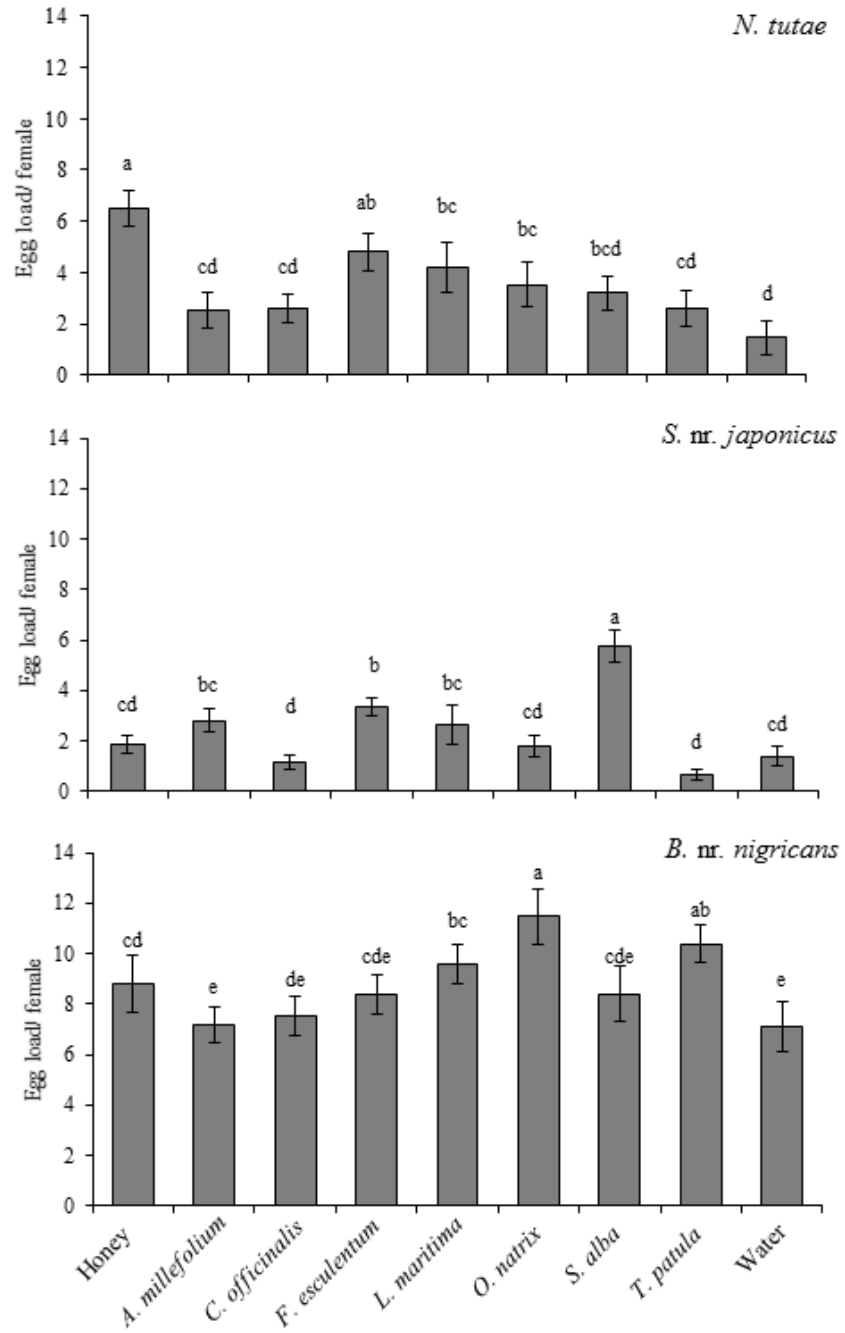
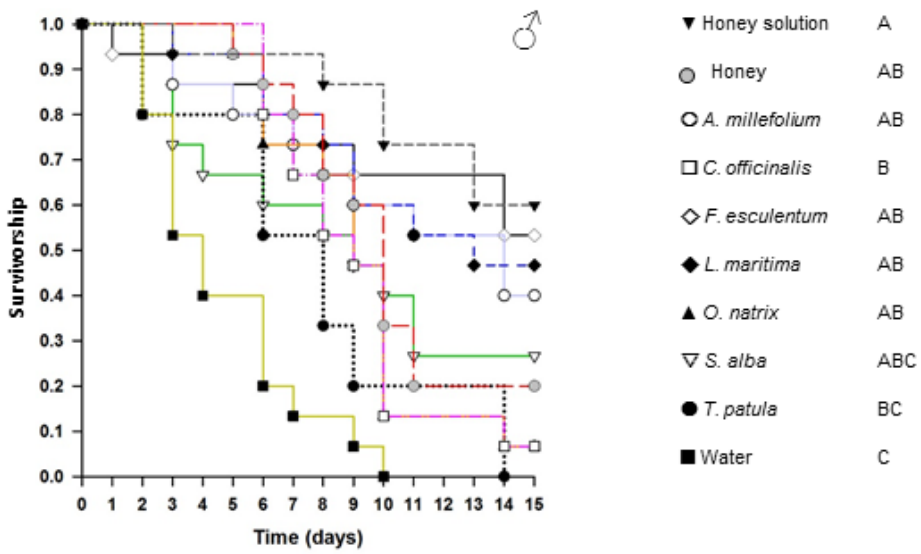
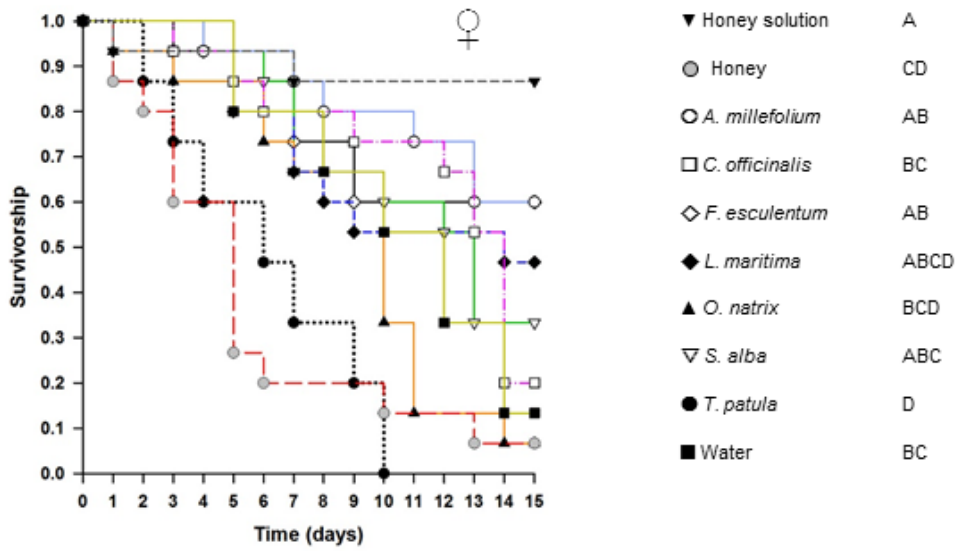


Figure 5



496 Table 1.

	<i>N. tutae</i>				<i>S. nr. japonicus</i>				<i>B. nr. nigricans</i>			
	Female		Male		Female		Male		Female		Male	
	χ^2	P	χ^2	P	χ^2	P	χ^2	P	χ^2	P	χ^2	P
Honey - <i>A. millefolium</i>	4,9460	0,0262	4,3050	0,0380	1,6723	0,1960	0,6618	0,4159	15,5106	<0,0001	8,4758	0,0036
Honey - <i>C. officinalis</i>	12,4881	0,0004	16,3039	<0,0001	4,2420	0,0394	14,6002	0,0001	21,2322	<0,0001	11,4816	0,0007
Honey - <i>F. esculentum</i>	4,8933	0,0270	3,5564	0,0593	6,2615	0,0123	0,0544	0,8155	0,9843	0,3211	2,7558	0,0969
Honey - <i>L. maritima</i>	0,3275	0,5671	0,0573	0,8108	0,1698	0,6803	1,6920	0,1933	10,5945	0,0011	0,8307	0,3621
Honey - <i>O. natrix</i>	13,2803	0,0003	18,7788	<0,0001	16,5944	<0,0001	14,9057	0,0001	29,4694	<0,0001	10,939	0,0009
Honey - <i>S. alba</i>	4,8309	0,0280	13,5477	0,0002	12,1212	0,0005	6,4376	0,0112	19,9996	<0,0001	11,4363	0,0007
Honey - <i>T. patula</i>	10,8711	0,0010	13,4945	0,0002	12,1597	0,0005	5,0105	0,0252	26,3983	<0,0001	12,1597	0,0005
Honey - Water	13,6547	0,0002	15,4478	<0,0001	9,2110	0,0024	3,5008	0,0613	22,3406	<0,0001	12,5326	0,0004
<i>A. millefolium</i> - <i>C. officinalis</i>	4,0103	0,0452	2,9239	0,0873	1,2318	0,2671	8,5340	0,0035	1,7339	0,1879	2,4016	0,1212
<i>A. millefolium</i> - <i>F. esculentum</i>	0,3087	0,5785	0,2118	0,6454	1,9871	0,1586	0,8625	0,3530	18,5181	<0,0001	28,8499	<0,0001
<i>A. millefolium</i> - <i>L. maritima</i>	9,8513	0,0017	3,7183	0,0538	3,0075	0,0829	1,9935	0,158	0,0034	0,9533	7,8375	0,0051
<i>A. millefolium</i> - <i>O. natrix</i>	2,9112	0,0880	5,1901	0,0227	8,9939	0,0027	10,0504	0,0015	7,1660	0,0074	1,3017	0,2539
<i>A. millefolium</i> - <i>S. alba</i>	0,0278	0,8676	1,3620	0,2432	5,0299	0,0249	2,0709	0,1501	0,5199	0,4709	2,0320	0,1540
<i>A. millefolium</i> - <i>T. patula</i>	0,8346	0,3609	1,7109	0,1909	5,5594	0,0184	3,2760	0,0703	1,8748	0,1709	2,8460	0,0916
<i>A. millefolium</i> - Water	3,3165	0,0686	2,5611	0,1095	4,0572	0,0440	1,6356	0,2009	3,6245	0,0569	7,9069	0,0049
<i>C. officinalis</i> - <i>F. esculentum</i>	11,1698	0,0008	8,0407	0,0046	0,3940	0,5302	13,9151	0,0002	22,0025	<0,0001	32,3897	<0,0001
<i>C. officinalis</i> - <i>L. maritima</i>	22,3602	<0,0001	17,8407	<0,0001	6,948	0,0084	14,1072	0,0002	0,2352	0,6277	13,0107	0,0003
<i>C. officinalis</i> - <i>O. natrix</i>	0,0103	0,9190	0,9835	0,3213	0,0605	0,8057	0,0005	0,9815	1,8413	0,1748	0,0247	0,8751
<i>C. officinalis</i> - <i>S. alba</i>	4,1237	0,0423	1,1692	0,2796	0,1466	0,7018	0,8346	0,3609	0,3974	0,5284	0,0116	0,9143
<i>C. officinalis</i> - <i>T. patula</i>	1,5247	0,2169	0,3095	0,5780	0,0376	0,8463	0,8759	0,3493	0,3200	0,5716	0,1389	0,7094
<i>C. officinalis</i> - Water	0,0588	0,8084	0,1029	0,7484	0,0327	0,8565	3,6868	0,0548	1,0817	0,2983	1,4553	0,2277
<i>F. esculentum</i> - <i>L. maritima</i>	8,2961	0,0040	3,9717	0,0463	7,0518	0,0079	0,5799	0,4463	14,3314	0,0002	9,2944	0,0023
<i>F. esculentum</i> - <i>O. natrix</i>	19,7238	<0,0001	10,2025	0,0014	7,5397	0,0060	15,2557	<0,0001	28,2934	<0,0001	30,3483	<0,0001
<i>F. esculentum</i> - <i>S. alba</i>	0,1012	0,7504	4,2805	0,0386	2,5384	0,1111	5,9472	0,0147	22,6588	<0,0001	31,5047	<0,0001

<i>F. esculentum</i> - <i>T. patula</i>	9,8366	0,0017	3,7627	0,0524	2,9966	0,0834	6,0117	0,0142	25,9473	<0,0001	33,0611	<0,0001
<i>F. esculentum</i> - Water	22,1186	<0,0001	5,2933	0,0214	0,7092	0,3997	6,4135	0,0113	23,4675	<0,0001	30,8586	<0,0001
<i>L. maritima</i> - <i>O. natrix</i>	26,5835	<0,0001	19,4992	<0,0001	13,0700	0,0003	13,1972	0,0003	1,2878	0,2564	12,4173	0,0004
<i>L. maritima</i> - <i>S. alba</i>	9,0224	0,0027	15,2644	<0,0001	11,1382	0,0008	8,8252	0,0030	0,2246	0,6355	11,1914	0,0008
<i>L. maritima</i> - <i>T. patula</i>	18,1925	<0,0001	12,9372	0,0003	11,4981	0,0007	7,836	0,0051	0,2692	0,6039	12,6588	0,0004
<i>L. maritima</i> - Water	28,0509	<0,0001	15,4478	<0,0001	9,8701	0,0017	7,4085	0,0065	1,4864	0,2228	18,4798	<0,0001
<i>O. natrix</i> - <i>S. alba</i>	4,8438	0,0278	3,4042	0,0650	0,7175	0,3970	1,4700	0,2254	4,2832	0,0385	0,0001	0,9942
<i>O. natrix</i> - <i>T. patula</i>	4,2604	0,0390	1,7443	0,1866	0,7384	0,3902	0,7643	0,3820	4,2890	0,0384	0,1717	0,6786
<i>O. natrix</i> - Water	0,4462	0,5041	0,8758	0,3494	3,3356	0,0678	3,8012	0,0512	0,0150	0,9027	1,6969	0,1927
<i>S. alba</i> - <i>T. patula</i>	1,8281	0,1764	0,0409	0,8397	0,0053	0,9422	0,0008	0,9770	0,3863	0,5343	0,296	0,5864
<i>S. alba</i> - Water	5,9094	0,0151	0,1806	0,6708	0,3708	0,5426	0,3294	0,5660	1,8952	0,1686	0,5981	0,4393
<i>T. patula</i> - Water	7,5982	0,0058	0,2905	0,5899	0,5728	0,4492	0,7242	0,3948	2,2561	0,1331	0,1877	0,6648

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	<i>T. absoluta</i>			
	Female		Male	
	χ^2	P	χ^2	P
Honey - Honey solution	19,8251	< 0,0001	5,8282	0,0158
Honey - <i>A. millefolium</i>	15,1129	0,0001	1,0204	0,3124
Honey - <i>C. officinalis</i>	9,7453	0,0018	1,1858	0,2762
Honey - <i>F. esculentum</i>	13,6703	0,0002	2,6572	0,1031
Honey - <i>L. maritima</i>	10,4539	0,0012	1,6421	0,2000
Honey - <i>O. natrix</i>	3,4531	0,0631	1,0157	0,3135
Honey - <i>S. alba</i>	9,7081	0,0018	0,0317	0,8587
Honey - <i>T. patula</i>	0,0534	0,8173	3,2969	0,0694
Honey - Water	6,6962	0,0097	16,4784	< 0,0001
Honey solution - <i>A. millefolium</i>	2,2252	0,1358	1,3519	0,2449
Honey solution - <i>C. officinalis</i>	10,7513	0,001	10,9601	0,0009
Honey solution - <i>F. esculentum</i>	2,2907	0,1302	0,2135	0,6440
Honey solution - <i>L. maritima</i>	4,7340	0,0296	0,8104	0,368
Honey solution - <i>O. natrix</i>	16,9302	< 0,0001	10,6028	0,0011
Honey solution - <i>S. alba</i>	7,5747	0,0059	4,7985	0,0285
Honey solution - <i>T. patula</i>	21,6580	< 0,0001	14,4466	0,0001
Honey solution - water	13,4873	0,0002	24,6578	< 0,0001
<i>A. millefolium</i> - <i>C. officinalis</i>	2,7989	0,0943	3,4501	0,0632
<i>A. millefolium</i> - <i>F. esculentum</i>	0,0265	0,8706	0,4309	0,5115
<i>A. millefolium</i> - <i>L. maritima</i>	0,7318	0,3923	0,1034	0,7478
<i>A. millefolium</i> - <i>O. natrix</i>	10,0072	0,0016	3,2205	0,0727
<i>A. millefolium</i> - <i>S. alba</i>	2,0548	0,1517	1,0250	0,3113
<i>A. millefolium</i> - <i>T. patula</i>	19,8196	< 0,0001	6,2825	0,0122
<i>A. millefolium</i> - Water	5,4014	0,0201	15,4066	< 0,0001
<i>C. officinalis</i> - <i>F. esculentum</i>	2,4219	0,1196	5,8475	0,0156
<i>C. officinalis</i> - <i>L. maritima</i>	0,6503	0,4200	4,4856	0,0342
<i>C. officinalis</i> - <i>O. natrix</i>	4,6962	0,0302	0,0047	0,9451
<i>C. officinalis</i> - <i>S. alba</i>	0,0018	0,9665	0,4272	0,5134
<i>C. officinalis</i> - <i>T. patula</i>	15,5783	< 0,0001	1,1048	0,2932
<i>C. officinalis</i> - Water	0,9724	0,3241	12,8736	0,0003
<i>F. esculentum</i> - <i>L. maritima</i>	0,5729	0,4491	0,1177	0,7315
<i>F. esculentum</i> - <i>O. natrix</i>	5,8233	0,0158	5,7503	0,0165
<i>F. esculentum</i> - <i>S. alba</i>	1,3536	0,2447	2,4240	0,1195
<i>F. esculentum</i> - <i>T. patula</i>	12,8025	0,0003	9,3806	0,0022
<i>F. esculentum</i> - Water	3,7899	0,0516	16,1817	< 0,0001
<i>L. maritima</i> - <i>O. natrix</i>	3,0791	0,0793	4,286	0,0384
<i>L. maritima</i> - <i>S. alba</i>	0,2045	0,6512	1,7640	0,1841
<i>L. maritima</i> - <i>T. patula</i>	9,328	0,0023	8,0187	0,0046
<i>L. maritima</i> - Water	1,6173	0,2035	17,8840	< 0,0001
<i>O. natrix</i> - <i>S. alba</i>	3,6265	0,0569	0,3578	0,5497
<i>O. natrix</i> - <i>T. patula</i>	7,6849	0,0056	1,4105	0,2350
<i>O. natrix</i> - Water	1,5806	0,2087	12,7145	0,0004

<i>S. alba</i> - <i>T. patula</i>	12,8679	0,0003	1,9081	0,1672
<i>S. alba</i> - Water	0,7357	0,3910	8,5525	0,0035
<i>T. patula</i> - Water	11,8196	0,0007	5,0386	0,0248

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