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1 **Lure and starve: host root exudates to suppress field**
2 **populations of cyst nematodes**

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15

16 **Abstract:** Plant parasitic nematodes are a burden to global food security, accounting for
17 substantial yield losses in agricultural production worldwide. Increasing concerns over the
18 environment and health issues have led to diminishing control options at the disposal of
19 agricultural producers. Lure and starve, a strategy that is based on stimulating hatching of
20 second stage juveniles (J2) of some cyst forming plant parasitic nematodes in the absence of
21 their host plant seems a promising approach to maintain sustainability in crop production. Here
22 we examined *in-situ* hatching of encysted eggs of the potato cyst nematode *Globodera pallida*
23 and the carrot cyst nematode *Heterodera carotae* following exogenous applications of host root
24 exudates in repeated field experiments during autumn and spring seasons. Population densities
25 (viable J2 g⁻¹ of soil) were assessed prior and post-application of root exudates. Results showed
26 hatching induction of up to 83% for *G. pallida* and 54% for *H. carotae* in field plots drenched
27 with their respective host root exudates. Spring season experiments were characterised by
28 limited soil moisture and did not reduce nematode population densities for *G. pallida*. The
29 potential for host root exudates in suppressing field population of cyst nematode in lure and
30 starve strategies looks promising for both cyst nematode species investigated.

31

32 **Keywords:** field experiments; hatching; *Heterodera carotae*; *Globodera pallida*; root
33 exudates.

34

35 **1. Introduction**

36 Cyst nematodes account for substantial annual yield losses and are second on the list of
37 economic importance in crop production, after root knot nematodes (Jones et al., 2013). Recent
38 political tensions over climate changes, increased awareness and health concerns have resulted
39 in diminishing options of phytochemicals available for pest and disease control. This has left
40 farmers with limited or no options in certain cases and pest and disease pressure risk worsening.

41 Within the European Union, annual losses associated to potato cyst nematodes (PCN)
42 are estimated at 9% in marketable potato tuber yield (Turner and Subbotin, 2013). The two
43 PCN species, *Globodera pallida* and *G. rostochiensis*, are placed under the A2 list of plant
44 parasitic nematodes recommended by EPPO (European and Mediterranean Plant Protection
45 Organisation) to member countries for regulation as quarantine pest in the national
46 phytosanitary regulations (Regulation (EU) 2016/2031). The strict application of these EU
47 quarantine requirements for fields detected with quarantine pests such as PCN further
48 complicates the situation and may increase the figures on losses associated to PCN in terms of
49 surface area under fallow or cultivated with an alternative crop. The use of resistant potato
50 cultivars has been very successful in suppressing multiplication of *G. rostochiensis*. The
51 dominant cultivation of *G. rostochiensis* resistant potato variety has in some cases led to the
52 selective presence of *G. pallida* in fields previously detected with mixed populations of PCN
53 (Evans and Haydock, 1990). Moreso, virulent populations of *G. pallida* have overcome resistant
54 cultivars as reported in Germany and in the Netherlands (Niere et al., 2014; Mwangi et al.,
55 2019).

56 The carrot cyst nematode (CCN) *Heterodera carotae* is a major phytosanitary problem
57 for many carrot producers, and has been reported in many European countries (Gautier et al.,
58 2019; Mugniéry and Bossis, 1988; Osborne, 1971), Canada (Yu et al., 2017), the USA (Berney
59 and Bird, 1992), Mexico (Escobar-Avila et al., 2018) and South Africa (Subbotin et al., 2010).

60 In the absence of the implementation of effective control methods, yield losses due to the carrot
61 cyst nematode *H. carotae* can reach 80% (Greco et al., 1993; Yu et al., 2017). Since the
62 withdrawal of 1,3-dichloropropene, a soil fumigant, coupled with the absence of resistant carrot
63 cultivars to *H. carotae*, the carrot sector has been left with no alternative solutions to control
64 this nematode species. This therefore highlights the urgency for alternative solutions to
65 maintain the competitiveness of the carrot sector with other vegetable crop.

66 The principles underlying hatching of cyst nematodes were recently illustrated and
67 discussed under controlled conditions (Ngala et al., 2021). However, Ngala et al. (2021) focused
68 on homogeneous cysts population originating from glasshouse cultures. Satisfactory results
69 from glasshouse studies by Ngala et al. (2021) highlighted the importance of field experiments
70 to observe the feasibility of the lure and stave strategy as an IPM component for cyst nematodes.

71 The evaluation of cyst nematode hatching is much more complex under field conditions.
72 The complexity of field situations is largely attributed to the heterogeneity in age, size and
73 viable juvenile content of cysts as well as the variability in the localization of infestation foci
74 (Schomaker and Been, 1999) amongst other natural and technical aspects such as the
75 positioning of cores during different sampling periods. Been et al. (2019) observed increases in
76 population densities between samples collected after host and non-host crops and associated
77 these increases to the variation of infestation foci between different sampling periods. These
78 factors require careful consideration when setting up field experiments on cyst nematode
79 infested fields.

80 Here we examined the effect of exogenous soil application of crude root exudates from
81 host plants on the population densities of two cysts nematodes species, *G. pallida* and *H.*
82 *carotae*, under field conditions in two different periods, autumn and spring seasons. To assess
83 the individual or cumulative effect of each root exudate, nematodes population densities were
84 precisely monitored in micro-plots experiments with complete randomised block designs.

85 **2. Materials and methods**

86 Experiments with potato cyst nematode (PCN) *G. pallida* were conducted between autumn
87 2020 and summer 2021 under field conditions in one of the French main Atlantic coast islands,
88 Noirmoutier. Experiments with *H. carotae* were conducted between autumn 2019 and summer
89 2020 under field conditions in the west coast of Normandy, France, near Créances. Soil samples
90 collected from experimental field sites were processed according to the norms laid out in NF
91 ISO 11464, autoclaved at 120°C and sent to Auréa Agrosociences (Ardon, France) to determine
92 particle size distribution, pH and organic matter content. Sentek Drill & Drop data loggers
93 (Sentek Technology, Agralis Services, Agen, France) were installed to record soil temperature
94 up to 30 cm soil depth at each experiment site. Local precipitations and atmospheric
95 temperature data were retrieved from Historique-Meteo.net ([https://www.historique-](https://www.historique-meteo.net)
96 [meteo.net](https://www.historique-meteo.net)).

97 **2.1 Root exudate production**

98 The first batch of potato root exudates were produced in February 2020 and a second batch was
99 produced in February 2021 using the susceptible *Solanum tuberosum* cv. Désirée. The
100 production of potato root exudates followed the same procedures as previously described in
101 Ngala et al. (2021). Briefly, sprouted tubers were suspended on tap water in plastic boxes such
102 that roots produced by the tubers were immediately immersed in water. The setup was placed
103 in the dark at 20°C ($\pm 0.5^\circ\text{C}$) and monitored over 21 days, before the water was collected and
104 adjusted such that one tuber was equivalent to 250 mL of exudate.

105 The carrot cultivar Touchon was selected as a previous *in vitro* screening showed that
106 root exudate from this cultivar was the best among 32 exudates from different Apiaceae (Ngala
107 et al., 2021), while a second cultivar, Maestro, not tested previously was added. For each carrot
108 cultivar (Touchon and Maestro), root exudates were produced two times, in September 2019

109 and in March 2020, by soil leaching using 528 pots each filled with 2 L of field collected soil
110 and placed in glasshouse regulated at 17/21°C night/day temperatures. Seven carrot seedlings
111 were transplanted in each pot. The collection of root exudates began four weeks post-
112 transplanting and continued at a weekly interval up to week-sixth post-transplanting, resulting
113 into a total of approximately 750 L after three successive collections for each carrot cultivar.

114 After production, root exudates were stored refrigerated at 4°C until required for
115 application within 4 to 5 months of production, otherwise, the root exudates were stored at -
116 40°C prior to use if an extended waiting period was necessary prior to application. Aliquots
117 were taken from each batch of root exudates and assessed for *in-vitro* hatch stimulation, using
118 twelve similar sized cysts of the corresponding nematode species per exudate as described in
119 Ngala et al. (2021), to confirm efficiency after the storage period prior to application on field
120 plots as compared with water (data not shown).

121 **2.2 Experimental design**

122 For *G. pallida*, two experiments were established in September 2020 (GpExpt-1 and GpExpt-
123 2) at separate locations in Noirmoutier, France. Another experiment (GpExpt-3) was then
124 established adjacent to the location of GpExpt-1 to observe the effect of root exudates on PCN
125 hatching during the spring season of 2021. The distribution of PCN on field plots was relatively
126 uniform (Supplementary Fig. S1), as the plots were used for the monoculture of potatoes in
127 previous years. Therefore, there was no evidence to contradict the choice of blocking treatments
128 on potato rows. As such, micro-plots (measuring 2 m * 0.7 m) representing individual replicates
129 were assigned to five potato rows, with each row containing three treatments arranged in
130 random blocks, separated by 1 m buffer zone in-between treatments on the same row (Fig. 1).
131 The treatments included root exudates diluted at 1:10 v/v (10%) exudates to water respectively
132 (T1), tap water (T2) and undiluted (100%) root exudates (T3). With three treatments and five

133 replicates, the randomized block design provides eight residual degrees of freedom for each *G.*
134 *pallida* experiment.

135 For *H. carotae*, two experiments were established in autumn 2019 and in spring 2020
136 to evaluate the effect of root exudates from two carrot cultivars (cv. Touchon and cv. Maestro)
137 on field hatching of *H. carotae*. The first experiment (HcExpt-1) had applications of root
138 exudates in autumn 2019, whereas the second experiment (HcExpt-2) had two series of
139 applications: autumn 2019 and a follow-up in spring 2020 to evaluate possible additional effect.
140 Micro-plots (4 m * 1.5 m) representing individual replicates were assigned to six carrot beds
141 with each bed containing three treatments arranged as random blocks, with a 1 m buffer zone
142 in-between treatments. The distribution of cysts over micro-plots was a little less uniform for
143 *H. carotae* than for *G. pallida* (Supplementary Fig. S2). Each treatment had six replicates, and
144 both experiments followed the same design. The treatments included tap water (W), undiluted
145 root exudates of carrot cv. Maestro (M) or cv. Touchon (T) (Fig. 2). With three treatments and
146 six replicates, the randomized block design provides 10 residual degrees of freedom for each
147 *H. carotae* experiment.

148 **2.3 Initial (P_i) and final (P_f) population densities**

149 For each micro-plot representing individual replicates, three plastic pegs were inserted along
150 the middle of the beds at an equidistance (of 0.5 m apart for *G. pallida* and of 1 m apart for *H.*
151 *carotae*). Twenty soil cores were sampled around each peg within the top 20 cm depth using a
152 soil corer (diameter 2.5 cm) and placed into separately labelled plastic bags making three-point
153 sub-samples of approximately 600 g each for each micro-plot and was used for population
154 densities estimates.

155 Soil samples from experiments with *G. pallida* were air-dried at 20°C, passed via a 2
156 mm mesh inox sieve and homogenised before 100 g was pulled and extracted through an

157 automated Seinhost elutriator. The extracted cyst collected on a filter paper were sorted from
158 organic debris under a binocular stereomicroscope and counted. After sorting, the cysts were
159 then crushed on a piece of aluminium block with the aid of a glass slide. The crushed cysts/eggs
160 mixed was rinsed into a Pyrex beaker and suspended into 80 mL of tap water. The suspension
161 was homogenised on a magnetic stirrer at 400 rpm before three aliquots of 1 mL were pulled
162 with a 10 mL glass pipette onto nematode counting slides for the quantification of viable and
163 non-viable eggs and juveniles (J2) to determine the population densities.

164 *Heterodera carotae* cysts were extracted from 300 g of air-dried (20°C) soil with the
165 aid of a Kort elutriator. The airdried mix of cysts and organic debris was crumbled onto a
166 crystallizing dish containing water and a strip of filter paper attached to the edge of the dish. A
167 drop of washing-up liquid was added in the centre of the crystallizing dish leading to the
168 attachment of the floated particles to the strip of filter paper. Cysts were then sorted from
169 organic debris under a binocular stereomicroscope and counted, before being crushed in a
170 Potter's tissue grinder containing a film of water. Crushed cysts were homogenised in 30 mL
171 of water before two aliquots of 5 mL were pulled onto a nematode counting slide for the
172 quantification of viable J2 and eggs under a stereomicroscope.

173 For each micro-plot on *G. pallida* or *H. carotae* experiments, the average of the three
174 point-samples represented the population density of the micro-plot at each time of assessment.
175 At the end of the experiment, the plots were sampled and assessed for nematode final population
176 densities (P_f) following the same procedures as described for P_i .

177 ***2.4 Species identification***

178 To identify the PCN species present in each experimental site, cysts were extracted from each
179 experimental block following P_i assessments to form a single sample, thus, five samples for
180 each experiment, from which subsamples were taken for DNA extraction after homogenisation

181 by handshaking. The PITSr3 primer for *G. rostochiensis* (amplicon size 434 bp) and PITSp4
182 primers for *G. pallida* (amplicon size 265 bp) (Bulman and Marshall, 1997) were used together
183 with the common primer ITS5 (White et al., 1990) in a multiplex polymerase chain reaction
184 (PCR).

185 Cysts collected in the experiments in the west coast of Normandy, France, were
186 considered to belong to the *H. carotae* species as a recent population genetics study showed
187 that at the west coast of Normandy, all individuals from 15 different fields (with 33 to 40
188 successfully genotyped individuals per population) were *H. carotae*, with no detection of mixed
189 population (with *H. carotae* and *H. cruciferae*) in the said locality (Esquibet et al., 2020).

190 *2.5 Applications of exudates*

191 Following soil sampling for initial *G. pallida* population density estimates (P_i), 10 L of each
192 treatment or water was spread over the micro-plots at establishment with the aid of a watering
193 can. The walking speed of the person applying the treatment was previously determined to
194 ensure that two passages matched with the volume of the watering can. The microplots were
195 flatten at the top and the hand application ensured that the applied quantity percolated into the
196 ridges, without running into the furrow. Treatments were repeated every three to four days over
197 the initial 15 days (five applications), thus each micro-plot received a total of 50 L of the same
198 treatment. The experimental plots were then left for a further 45 days, after which the plots were
199 re-sampled and analysed for *G. pallida* final population density (P_f).

200 For the *H. carotae* experiments, after the sampling for initial population density
201 estimates (P_i), 20 L of each exudate or tap water was spread over each micro-plot using a
202 watering can. The treatments were repeated every three to four days over the initial 20 days (six
203 applications), thus each micro-plot had 120 L of the same treatment. The experiments were then

204 left for an additional 30 days, after which the plots were re-sampled and analysed for *H. carotae*
205 final population density (P_f).

206 **2.6 Statistical analysis**

207 All statistical analyses were performed using the R software version 4.1.2 (R Core Team, 2021).
208 Normality of residuals and homogeneity of variances were checked by the Shapiro–Wilk and
209 the Levene tests, respectively. For each experiment, one-way ANOVA were performed to test
210 the treatment effect on the number of viable J2 per gram of soil for each assessment (P_i and P_f),
211 as well as the P_i vs. P_f effect for each treatment on the number of viable J2 per gram of soil.
212 Significant effects between treatments were determined using Tukey’s multiple range test (5%
213 significant level).

214

215 **3 Results**

216 **3.1 Field experiment characteristics: type of soil, temperature, precipitation and** 217 **nematode densities**

218 The soil at field sites where experiments were conducted on *G. pallida* and *H. carotae* were
219 both identified as sandy soils (Table 1). Soil pH was similar between the three fields, from 8.2
220 to 8.3, and the organic matter content was lower for the carrot cyst nematode (CCN)
221 experiments (1.2%) than for the PCN experiments (from 1.9 to 2.3%) (Table 1).

222 The cumulative precipitation during the autumn seasons was higher than precipitation
223 during spring seasons, especially during the period when the experiments were established, and
224 this was true between autumn 2019 and spring 2020 for *H. carotae*, and between autumn 2020
225 and spring 2021 for *G. pallida* (Table 2). During the autumn season of 2020, the average soil

226 temperature was higher at *G. pallida* experiment sites (from 12.9 to 14.6°C) than it was at the
227 *H. carotae* experiment sites during the autumn season of 2019 (from 9 to 11.9°C) (Table 2).

228 Multiplex PCR revealed that PCN extracted from soil at both field sites was the *G.*
229 *pallida* species. The build-up of nematode population over the years led to a mixture of cyst of
230 variable ages, sizes and shapes, although uniformly distributed over the field. Whatever the
231 nematode species and the experiment, there was neither treatment effect nor P_i vs. P_f effect on
232 the number of cysts (Supplementary Table S1 for *G. pallida* and Supplementary Table S2 for
233 *H. carotae*). The similarities in the number of cysts extracted between the two periods of
234 assessments (P_i and P_f) highlighted the quality of the sampling technique employed as well as
235 the uniformity in cyst distribution within blocks and individual replicates. This also showed
236 that, as expected, root exudates had no effect on the number of cysts, the relevant variables
237 being either the number of J2 g^{-1} of soil or the number of J2 cyst $^{-1}$. For the *H. carotae*
238 experiments, the initial population density (P_i) ranged between 3 to 5 J2 g^{-1} of soil on average,
239 whereas *G. pallida* experiment sites for autumn had P_i of between 35 to 40 J2 g^{-1} of soil at
240 GpExpt-1 and 40 to 50 J2 g^{-1} of soil at GpExpt-2 sites on average, respectively. The spring 2021
241 experiment (GpExpt-3) was positioned adjacent to GpExpt-1 and had a P_i density between 12
242 to 14 J2 g^{-1} of soil.

243 **3.2 Effect of root exudates on *G. pallida* hatching**

244 Assessments conducted 45 days after the final application of root exudates in autumn 2020 at
245 GpExpt-1 had a natural decline (tap water treatment) of about 38% from the initial PCN density,
246 while 72% and 83% hatch were observed on plots drenched with Desirée root exudates at 10%
247 and 100% concentrations, respectively (Fig. 3). Moreover, there was a significant treatment
248 effect on the final population ($F_{2,12} = 33.06$; $P < 0.0001$), with a *G. pallida* density lower in
249 plots drenched with exudates than in plots drenched with water (Fig. 3).

250 The natural decline at GpExpt-2 during the same period (autumn 2020) was around 15%
251 on control plot, while 63% and 70% hatch was observed on plots drenched with root exudates
252 at 10% and 100% concentrations, respectively (Fig. 4). As in GpExpt-1, the treatment effect
253 was significant in the final population ($F_{2,12} = 14.44$; $P = 0.0006$), with a *G. pallida* density
254 lower in plots drenched with exudates than in plots drenched with water (Fig. 4).

255 For GpExpt-3 conducted during the spring season of 2021, there was no treatment effect,
256 neither for the initial population ($F_{2,12} = 0.226$; $P = 0.801$) nor for the final population ($F_{2,12} =$
257 0.158 ; $P = 0.856$). The P_i vs. P_f comparisons were also not significant (Fig. 5).

258 **3.3 Effect of root exudates on *H. carotae* hatching**

259 For the HcExpt-1 conducted in autumn 2019, there was no treatment effect, neither for the
260 initial population ($F_{2,15} = 0.523$; $P = 0.603$) nor for the final population ($F_{2,15} = 0.095$; $P =$
261 0.910). The P_i vs. P_f comparisons were also not significant ($P > 0.05$), but the natural population
262 decline of 39% in plots drenched with water was smaller than the decline of *H. carotae* densities
263 observed in plots drenched with exudates: 42% and 46% for Maestro and Touchon root
264 exudates, respectively (Fig. 6).

265 A follow-up application of root exudates in the spring season of 2020 (HcExpt-2)
266 revealed a significant treatment effect in the final population ($F_{2,15} = 9.326$; $P = 0.002$), with a
267 *H. carotae* density lower in plots drenched with exudates than in plots drenched with water.
268 Moreover, the P_i vs. P_f comparisons between the autumn 2019 initial population density and
269 the spring 2020 final population density were strong and significant: the decline of *H. carotae*
270 density reached 51% and 54% in plots drenched with Maestro and Touchon root exudates,
271 respectively (Fig. 7).

272

273 **4. Discussion**

274 Experiments conducted under controlled conditions by Ngala et al. (2021) revealed the potential
275 of the application of root exudates to activate suicide hatching in the absence of the host plant
276 to reduce the population density of encysted juveniles in the soil. Moreover, it was apparent
277 from the glasshouse observations that soil moisture was an indispensable factor underpinning
278 hatching activity of encysted eggs of cyst nematodes. To test the suicide hatching strategy in
279 the field and to determine which window fits best for hatching stimulation of encysted eggs, we
280 investigated two possible windows, autumn and spring. Note that summer and winter seasons
281 have very hot and cold days, respectively, which are not suitable for encysted eggs hatching.
282 The present study therefore investigated the hatching activation of the potato cyst nematode
283 *Globodera pallida* under two autumn experiments and under a spring experiment in the fields.
284 Likewise, the carrot cyst nematode *Heterodera carotae* was investigated under an autumn
285 season experiment followed by spring season applications of the same treatments on the same
286 experiment to observe additional effects.

287 For *G. pallida*, the two autumn experiments were promising with very encouraging
288 reductions in juvenile densities as compared with control treatments. By contrast, when the
289 same setup was established in spring, the results were less encouraging, with no observed effect
290 of the treatment on the population density. The two seasons during which the field experiments
291 were performed had similar average monthly temperatures, but the spring season was dryer,
292 with very little rainfall during the experimental period than observations during the autumn
293 season according to the data retrieved from historique-meteo.net. Although soil humidity
294 probes were not installed at the field experiments, the observed difference according to the data
295 retrieved from historique-meteo.net confirmed previous glasshouse observations by Ngala et
296 al. (2021) on the necessity for adequate soil moisture and temperature for hatching of encysted
297 eggs.

298 For experiments with *H. carotae*, the climatic conditions in autumn 2019 seemed
299 favourable to the hatching stimulation, with similar rainfall and marginally lower than average
300 monthly temperatures compared with GpExpt-1 and GpExpt-2. However, the percentage of *H.*
301 *carotae* hatching during this period was relatively homogenous, with no statistical difference
302 either between treatments or between assessments (P_i vs. P_f) for individual treatments. By
303 contrast, follow-up treatments in the following spring (2020) revealed significant hatch
304 stimulation of *H. carotae* by host root exudates irrespective of the carrot cultivar used as
305 compared with water applications in rather more dry conditions.

306 The comparison between both cyst nematode species showed that hatching induction
307 was strong after a first stimulation by root exudate applications for *G. pallida* (83% reduction
308 in the autumn GpExpt-1), whereas the decrease of *H. carotae* population density was lower but
309 cumulative and became significant after two series of root exudate applications (54% reduction
310 in HcExpt-2). Such differences between both species may be explained by species specific
311 requirements in environmental/climatic conditions, and especially soil moisture and
312 temperature. Hatching is a complex combination of many biotic and abiotic factors specific to
313 each species. Fournet et al. (2018) showed strong differences in hatching behaviour between
314 different populations of the same species, *Heterodera schachtii*, according to different
315 experimental temperatures and cyst storage temperatures.

316 The influence of soil moisture on hatching of cyst nematodes has not been widely
317 studied, but, it is generally understood that at optimum soil temperatures, maximum cyst
318 nematodes hatching occurs when the soil moisture content is at field capacity (Masler and Perry,
319 2018). On the other hand, soil temperature has been investigated and most cyst nematodes show
320 optimum activity under a range from 10°C to 20°C with adequate soil moisture (Greco, 1981;
321 Greco and Brandonisio, 1986; Kaczmarek et al., 2014; Robinson et al., 1987). The conditions
322 recorded during the autumn season of 2020 falls within the optimum for PCN hatching, which

323 may explain the increased hatching observed in the field during this period. However, for *H.*
324 *carotae*, the late autumn period had an adequate soil moisture, but soil temperature for the
325 months of November and December during which the experiments were conducted may have
326 been too low to allow maximum hatching. Greco and Brandonisio (1986) observed hatching
327 rates of 31% for *H. carotae* in host root exudates at 10°C, which improved with increasing
328 temperature to an optimum at 20°C, beyond which hatching dropped sharply. The percentage
329 hatch observed for *H. carotae* in the present study during autumn falls within the range reported
330 by Greco and Brandonisio (1986). Follow-up application of root exudates in the following
331 spring season with relatively low cumulative precipitation accounted for a flash effect for *H.*
332 *carotae* showing significant reduction in population density.

333

334 Our results thus showed that the method could be effective in the field and for both species, but
335 the suicide hatching strategy faces several natural, technological and industrial challenges. On
336 the one hand, one could question whether suicide hatching efficiency could be improved with
337 repeated crude exudate applications to stimulate the hatching of the entire population of
338 juveniles contained in the cysts, which seems to be the case for *H. carotae* with the additional
339 effect observed herein. By contract, Devine and Jones (2000a) recommended the optimization
340 of the application techniques rather than additional applications for PCN because they noticed
341 that further applications of root exudate did not yield additional hatching as a portion of the
342 juveniles within cysts were less or non-receptive to root exudates at time of application. This
343 unhatched group of juveniles has been described in PCN species as belonging to the phase 3
344 hatching group, mainly constituted of dormant embryos and corresponding to essentially all the
345 unhatched eggs which are said to be in diapause (Masler et al., 2013; Masler and Rogers, 2011;
346 Sommerville and Davey, 2002). Specific conditions are required (cumulative time and
347 temperature) to attain the end of the diapause before the phase 3 group could resume hatching.

348 A second challenge with the suicide hatching strategy would be technical and industrial
349 on the required quantities of root exudates for field-scale application, as well as the technologies
350 needed for the identification, isolation and synthesis of active molecules (Devine and Jones
351 2000b, Masamune et al., 1982). The amount of crude exudates needed to treat large cropping
352 areas is clearly the main challenge to solve. The identification, isolation and synthesis of active
353 molecules involved in the hatching process might be an exciting and very promising option,
354 which could largely be supported by new metabolomic approaches and technologies. However,
355 it is a much complex situation considering that the hatching response of encysted eggs is
356 triggered by not one, but a blend of several substances including hatching factors (stimulators)
357 and hatching inhibitors (Byrne et al., 1998). Moreover, root exudates are composed of a wide
358 range of organic compounds of low molecular weight belonging to the primary (alcohols, amino
359 acids, organic acids, sugars, nucleic bases and nucleotides) or secondary metabolites (alkaloids,
360 phenylpropanoids, or terpenes) of the plant (Bais et al., 2006; Badri and Vivanco, 2009; Faure
361 et al., 2009; van Dam and Bouwmeester, 2016). These metabolites are not so easy to isolate,
362 identify (for their biological activity) and synthesis, thus may not be that cost effective.

363 A final question would centre around the durability of the suicide hatching strategy. The
364 use of crude root exudate is expected to be durable as the chemical signal used is the result of
365 a long co-evolution process between host plants and nematodes. Unlike products with a
366 nematicidal effect or resistant plant cultivars which may respectively select for resistant and
367 virulent individuals, the use crude root exudates would select for individuals that are unable to
368 hatch. Conversely, the use of 'synthetic' root exudates (i.e. exudates constituted of one or more
369 molecules identified through chemical analysis with a significant hatching stimulation effect)
370 could confer a poor durability potential on this strategy if the selected individuals are unable to
371 hatch with a subsequent application of the synthetic exudates but remain receptive to the
372 presence of the host plant when it is cultivated. Such hypothesis could be easily tested as some

373 molecules involved in the hatching of some cyst nematode species have now been identified
374 (for a review on the subject see Sikder and Vestergard, 2020), such as glycinoclepin A
375 involved in the hatching of *Heterodera glycines*, the soybean cyst nematode, or solanoeclepin
376 A (and now solanoeclepin B, Shimizu et al., 2023) involved in the hatching of the potato cyst
377 nematodes *G. pallida* and *G. rostochiensis*. There is no such data for the carrot cyst nematode.

378

379 To effectively replace the use of “synthetic” exudates, the use of trap crops or cover crops with
380 host related attributes that can stimulate hatching without allowing for multiplication might be
381 a useful alternative. Ngala et al. (2021) reported a wide list of host and nonhost species with
382 hatching activity for three cyst nematodes species including *H. carotae* and *G. pallida*. Trap
383 cropping for PCN management has been well documented, and has resulted in efficacy of up to
384 97% on field plots (Scholte 2000). However, their practical application needs careful
385 considerations prior to application, such as timing for sowing dates and destruction,
386 authorisation for their use within the locality and the disease problems within the field. These
387 considerations are necessary to ensure that solving one huddle would not be introducing a
388 potential worst-case scenario.

389

390 Suicide hatching on field plots has previously been reported for *G. rostochiensis* with the
391 application of tomato root leachates (Devine and Jones, 2000a). Results from the present study
392 has undoubtedly confirmed that the application of crude host root exudates can significantly
393 reduce the population densities of two more cyst nematodes species (*G. pallida* and *H. carotae*)
394 in field soils and has thus proven the effectiveness of this method for consideration into an IPM
395 program.

396

397 **Declaration of competing interest**

398 The authors declare that they have no known competing financial interests or personal
399 relationships that could have appeared to influence the work reported in this paper.

400

401 **Data availability**

402 Additional or supplementary data will be made available on request.

403

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409

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- 528

529 **Figure legends**

530

531 **Fig. 1:** Randomised block design and layout of micro-plots in the field experiments examining
532 the effect of root exudates on the hatching of *Globodera pallida*. Treatments include potato root
533 exudates at 10% (T1: patterned), tap water (T2: blue), and undiluted root exudates (T3: green).

534

535 **Fig. 2:** Randomised block design and layout of micro-plots in the field experiments examining
536 the effect of root exudates on the hatching of *Heterodera carotae*. Treatments included tap
537 water (W), root exudates of carrot cv. Maestro (M) and cv. Touchon (T).

538

539 **Fig. 3:** The population densities of *Globodera pallida* prior (pattern) and post (black)
540 application of potato root exudates for Experiment-1 conducted in Noirmoutier island during
541 autumn of 2020 (GpExpt-1). Error bars represent standard errors of means. Different letters
542 above bars with same case indicate significant differences between treatments (Tukey's test).
543 The P_i vs. P_f effect for individual treatments are indicated by the percent reduction of viable J2
544 g^{-1} of soil and its associated *P-value*.

545

546 **Fig. 4:** The population densities of *Globodera pallida* prior (pattern) and post (black)
547 application of potato root exudate for Experiment-2 conducted in Noirmoutier island during
548 autumn of 2020 (GpExpt-2). Error bars represent standard errors of means. Different letters
549 above bars with same case indicate significant differences between treatments (Tukey's test).
550 The P_i vs. P_f effect for individual treatments are indicated by the percent reduction of viable J2
551 g^{-1} of soil and its associated *P-value*.

552

553 **Fig. 5:** The population densities of *Globodera pallida* prior (pattern) and post (black)
554 application of potato root exudate for Experiment-3 conducted in Noirmoutier island during
555 spring 2021 (GpExpt-3). Error bars represent standard errors of means. Different letters above
556 bars with same case indicate significant differences treatments (Tukey's test). The P_i vs. P_f
557 effect for individual treatments are indicated by the percent reduction of viable J2 g^{-1} of soil
558 and its associated *P-value*.

559

560 **Fig. 6:** The population densities of *Heterodera carotae* prior (pattern) and post (black)
561 application of carrot root exudate for Experiment-1 conducted in Créances during autumn 2019
562 (HcExpt-1). Error bars represent standard errors of means. Different letters above bars with
563 same case indicate significant differences between treatments (Tukey's test). The P_i vs. P_f effect
564 for individual treatments are indicated by the percent reduction of viable J2 g^{-1} of soil and its
565 associated *P-value*.

566

567 **Fig. 7:** The population densities of *Heterodera carotae* prior (pattern) and post (black)
568 application of carrot root exudate for Experiment-2 conducted in Créances during autumn 2019
569 (P_i) and spring 2020 (P_f after two series of applications) (HcExpt-2). Error bars represent
570 standard errors of means. Different letters above bars with same case indicate significant
571 differences (Tukey's test). The P_i vs. P_f effect for individual treatments are indicated by the
572 percent reduction of viable J2 g^{-1} of soil and its associated *P-value*.

573

574

575 **Table 1.** Analysis for soil particle size distribution, pH and the organic matter (OM) contents
576 for the field experiments.

Field experiments	Sand (%)		Silt (%)		Clay (%)	OM (%)	pH
	Fine	Coarse	Fine	Coarse			
GpExpt-1 & 3	13.5	68	4.5	2.1	8.7	2.3	8.3
GpExpt-2	10.6	80.0	2.3	1.4	3.9	1.9	8.3
HcExpt-1 & 2	30.2	50.1	1.9	2.5	2.6	1.2	8.2

577

578

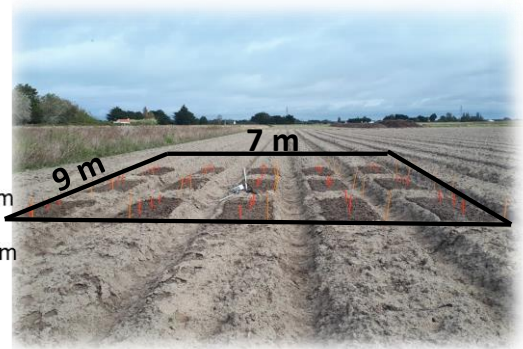
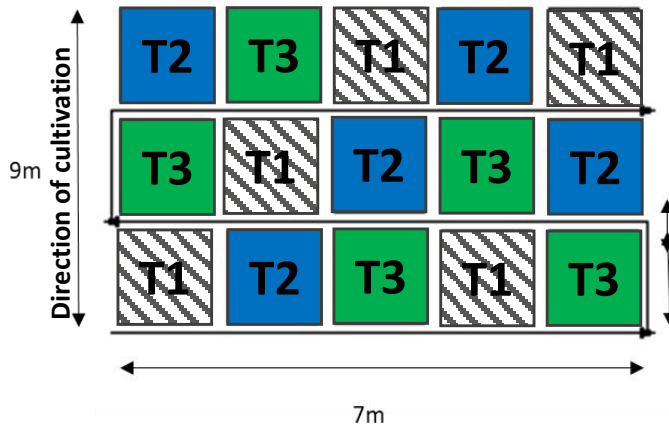
579 **Table 2.** Cumulative precipitation and average monthly soil temperatures over the duration of
580 the experiments conducted in the Autumn seasons of 2019 and 2020 as well as in the Spring
581 seasons of 2020 (*H. carotae*) and 2021 (*G. pallida*).

Experiment/ season	Period	Cumulative monthly precipitation (mm)	Average monthly soil temperature (°C)
<i>H. carotae</i> Autumn 2019	18 to 31 October	87.0	11.9
	01 to 30 November	145.5	9.0
	01 to 16 December	41.6	9.0
<i>H. carotae</i> Spring 2020	21 to 30 April	19.5	12.8
	01 to 31 May	19.5	14.0
	01 to 11 June	21.5	14.7
<i>G. pallida</i> Autumn 2020	24 to 30 September	19.2	14.6
	01 to 31 October	176.0	13.3
	01 to 18 November	60.4	12.9
<i>G. pallida</i> Spring 2021	17 to 31 March	5.6	12.1
	01 to 30 April	25.0	14.7
	01 to 12 May	18.3	15.3

582

583

584 Fig. 1 – Ngala et al

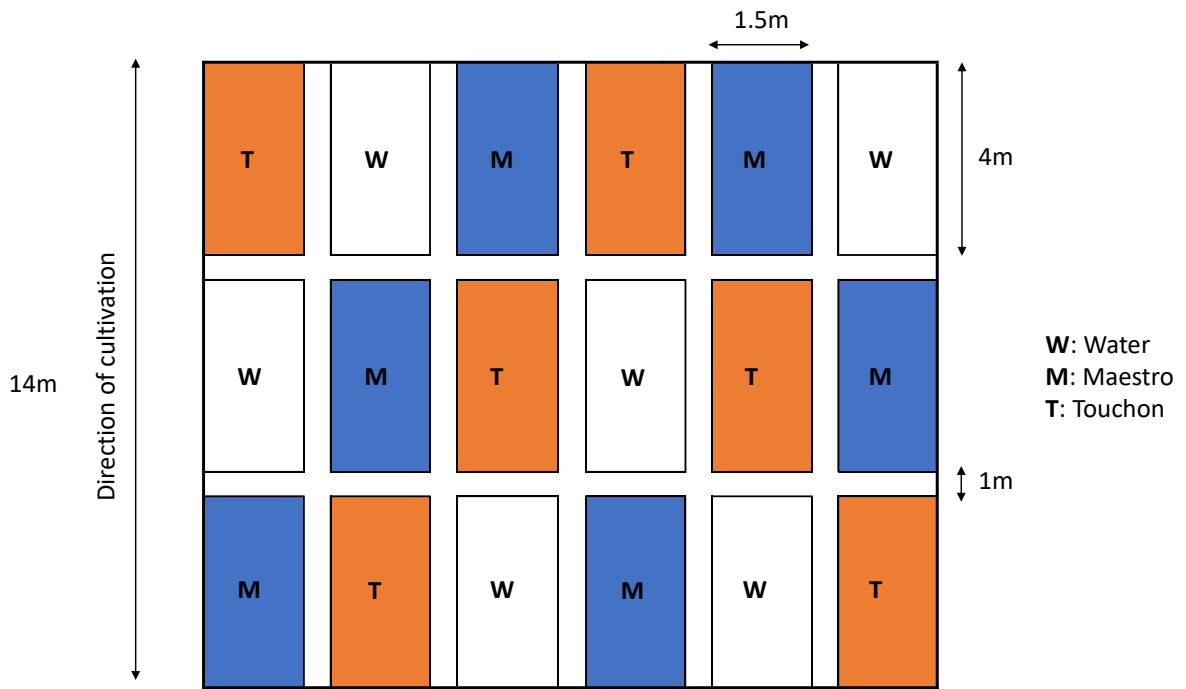


Field view of the micro-plots

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586

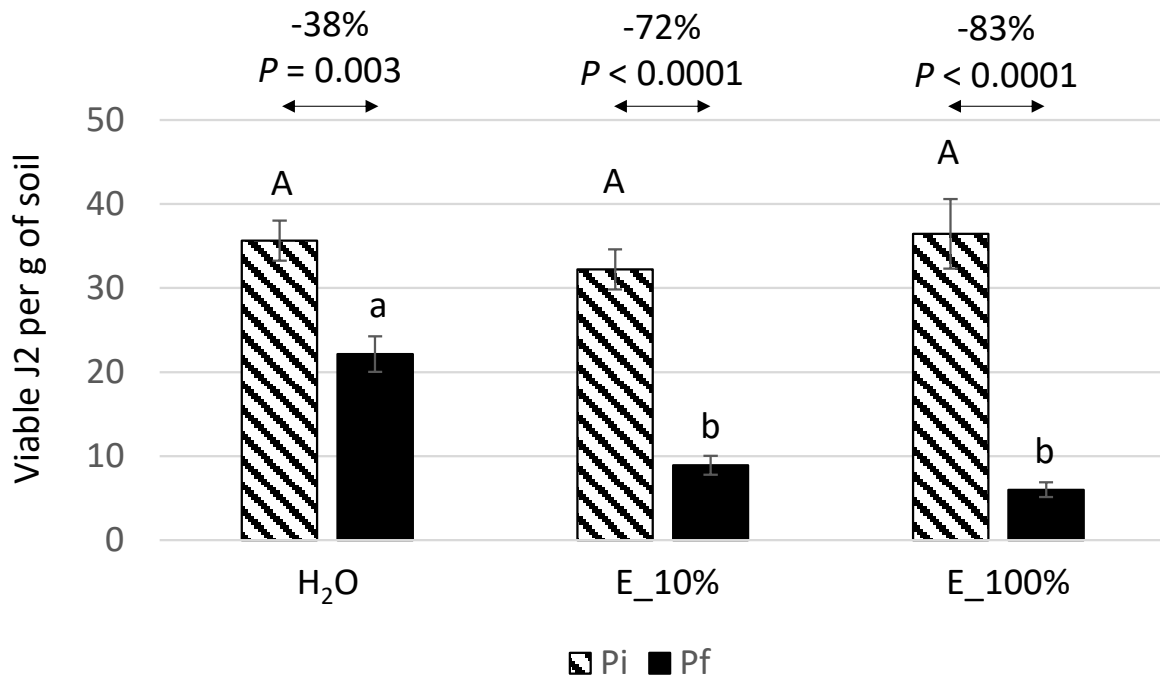
587 Fig. 2 – Ngala et al



588

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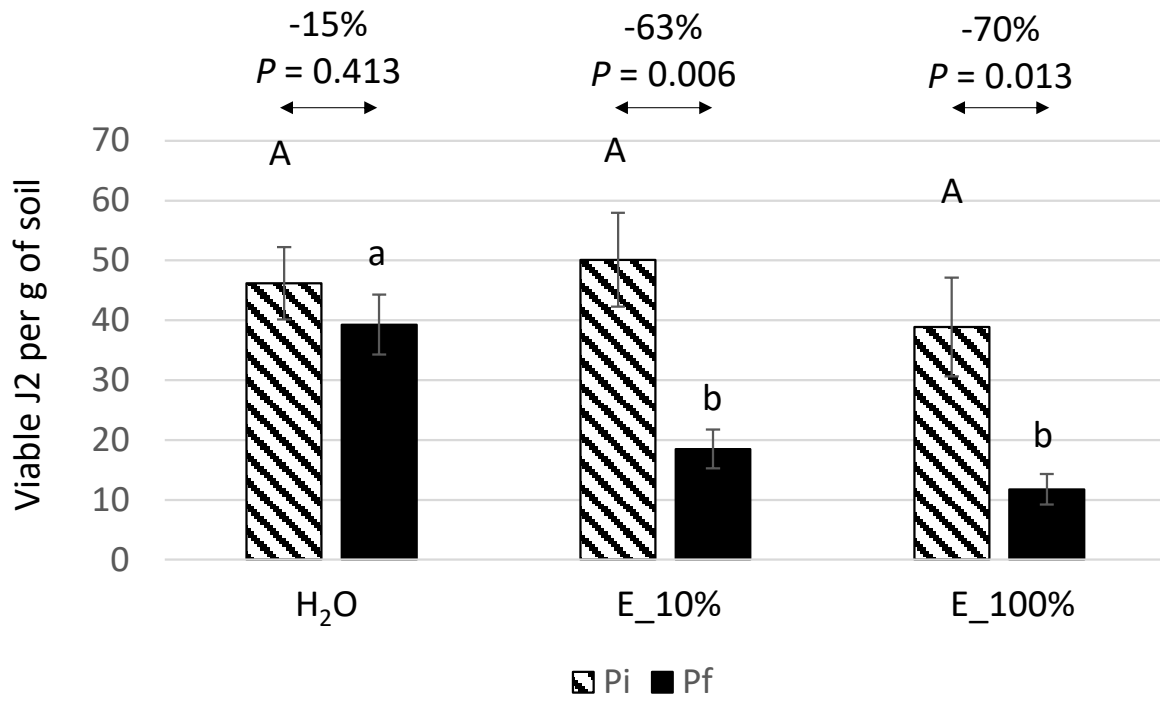
590 Fig. 3 – Ngala et al



591

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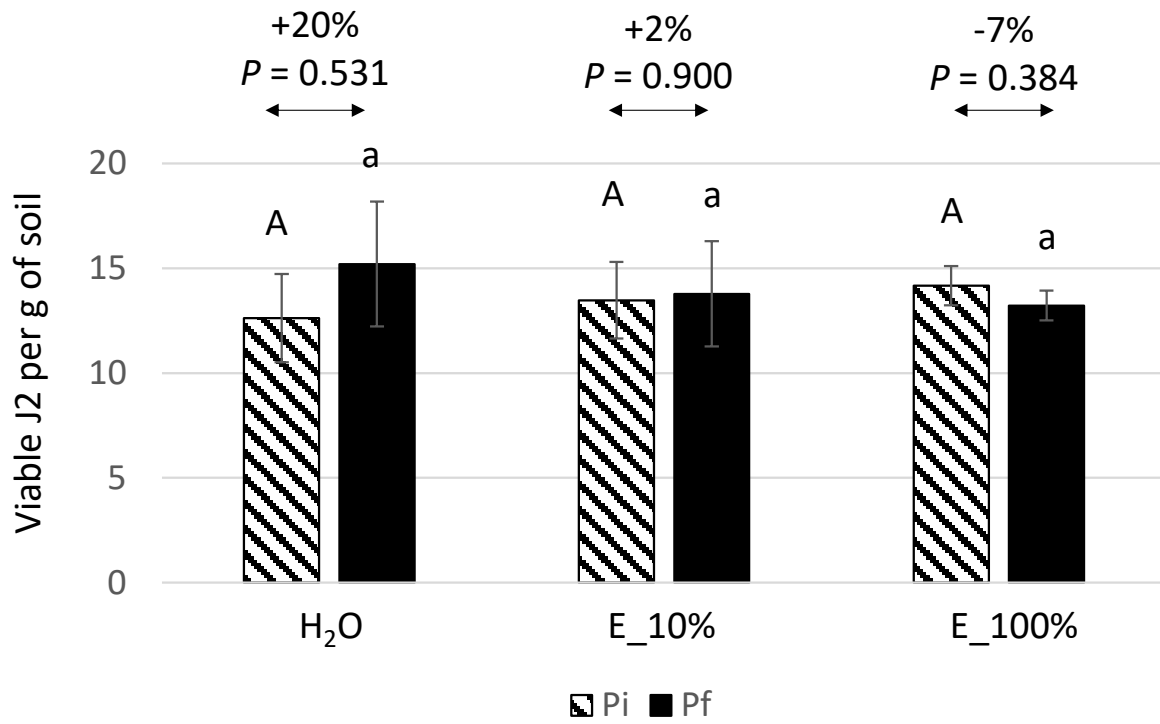
593 Fig. 4 – Ngala et al



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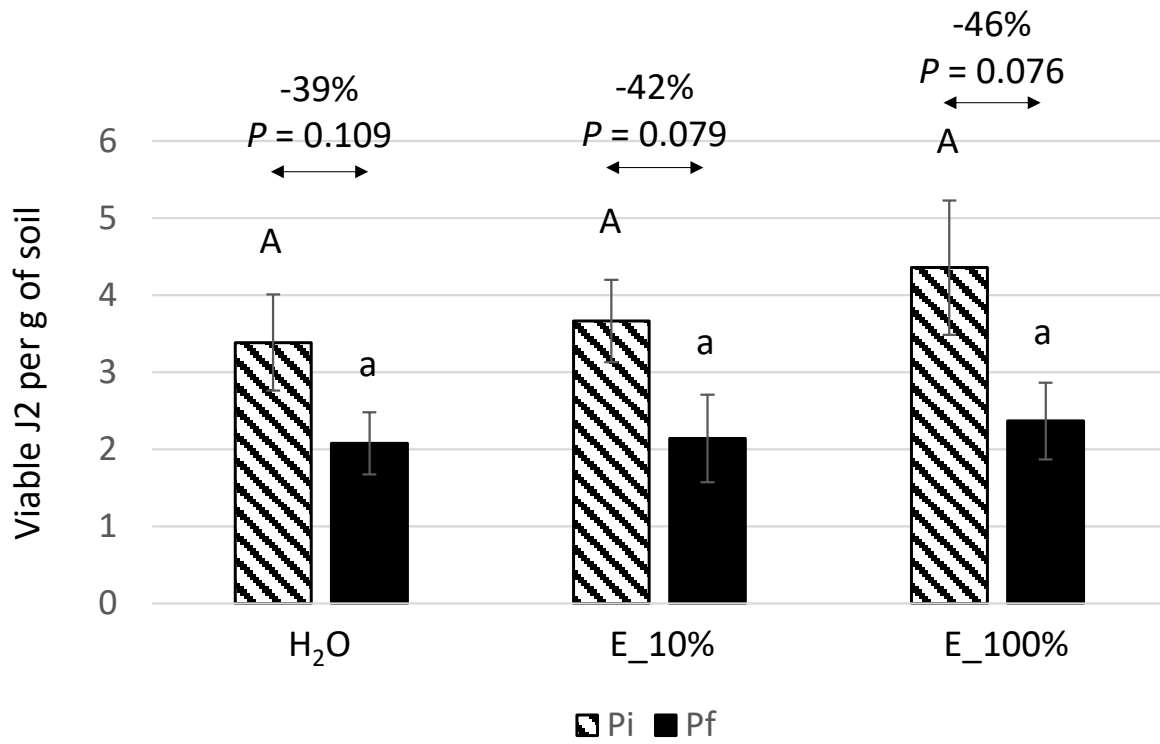
596 Fig. 5 – Ngala et al



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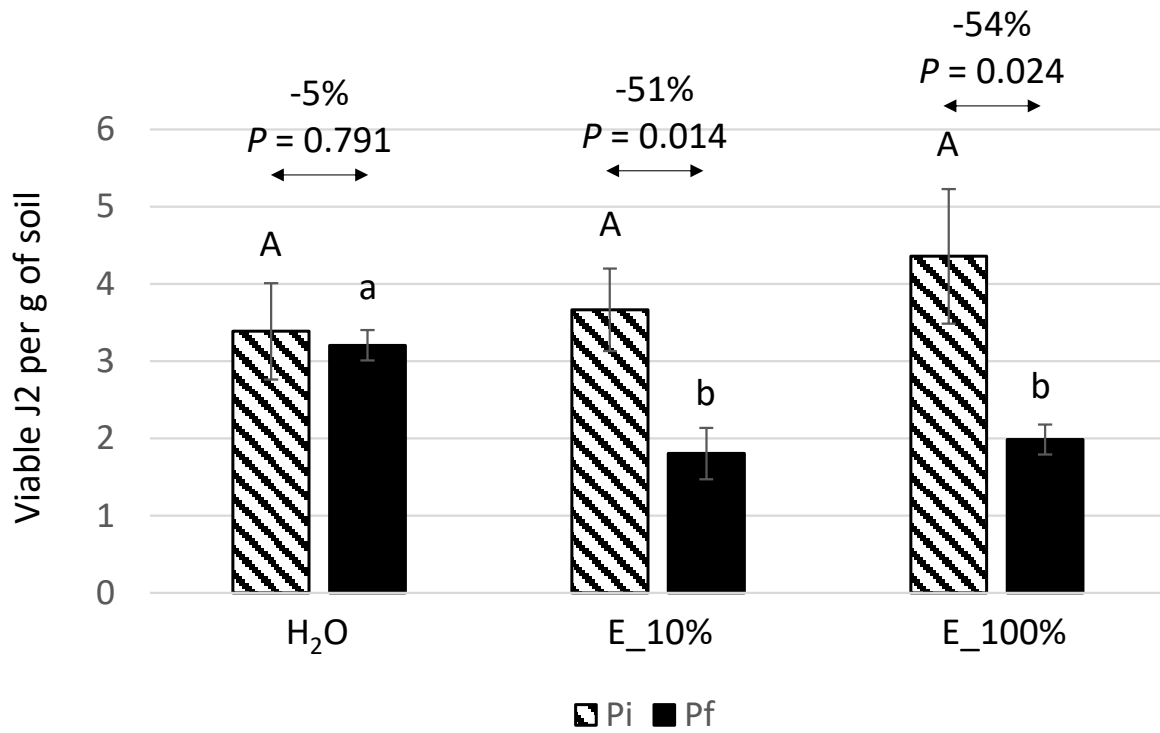
599 Fig. 6 – Ngala et al



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602 Fig. 7 – Ngala et al



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