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

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

1. Introduction

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

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

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

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

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

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

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

2. Materials and methods

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

2.1 Root exudate production

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

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

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

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

2.2 Experimental design

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

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

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

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

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

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

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

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

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

2.4 Species identification

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

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

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

2.5 Applications of exudates

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

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

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

2.6 Statistical analysis

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

3 Results

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

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

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

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

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

3.2 Effect of root exudates on G. pallida hatching

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

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

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

3.3 Effect of root exudates on H. carotae hatching

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

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

4. Discussion

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

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

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

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

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

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

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

Declaration of competing interest

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

Data availability

Additional or supplementary data will be made available on request.

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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

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

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

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

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)
H. carotae 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
H. carotae 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
G. pallida 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
G. pallida 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

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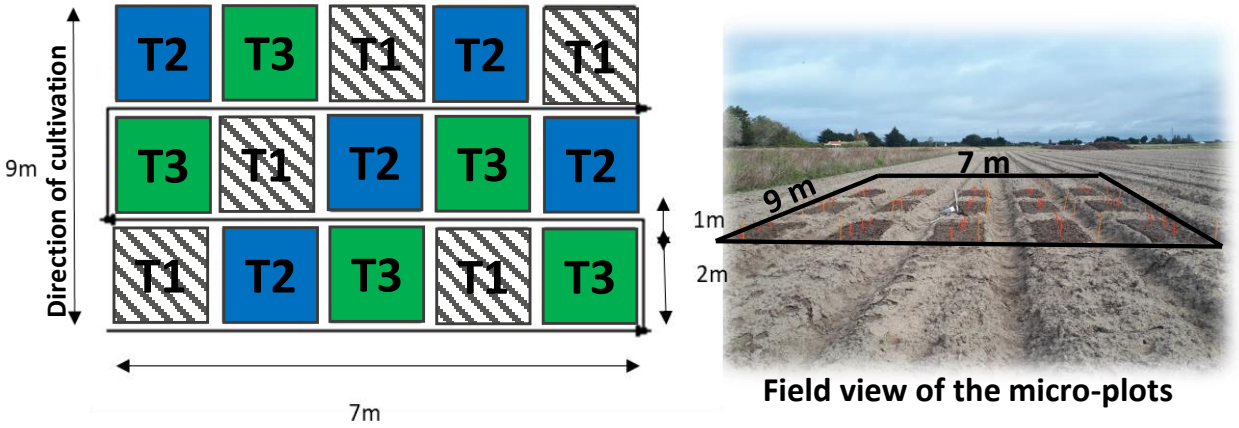
584 **Fig. 1 – Ngala et al**



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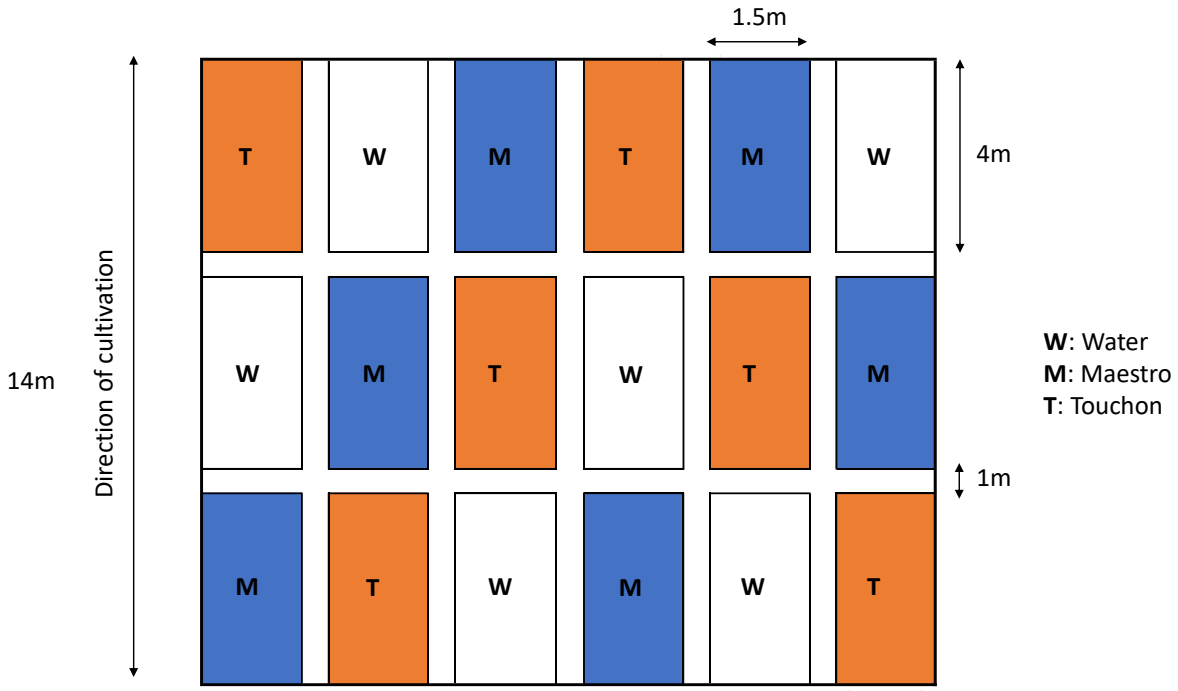
587 **Fig. 2 – Ngala et al**



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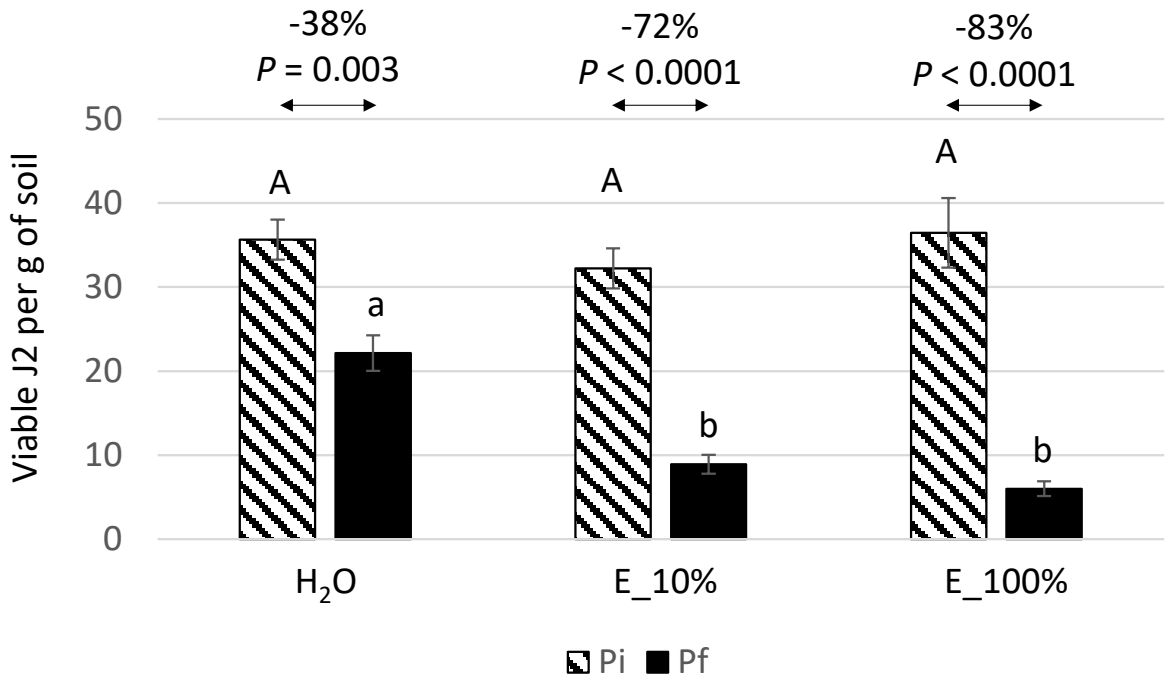
590 **Fig. 3 – Ngala et al**



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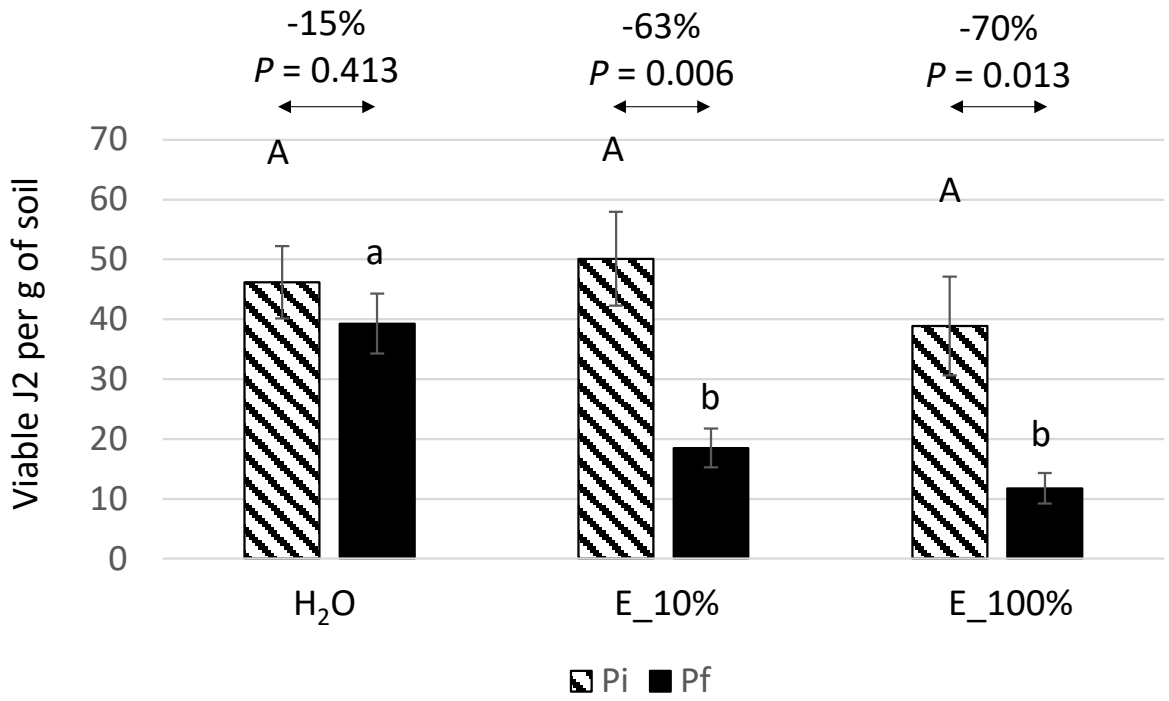
593 **Fig. 4 – Ngala et al**



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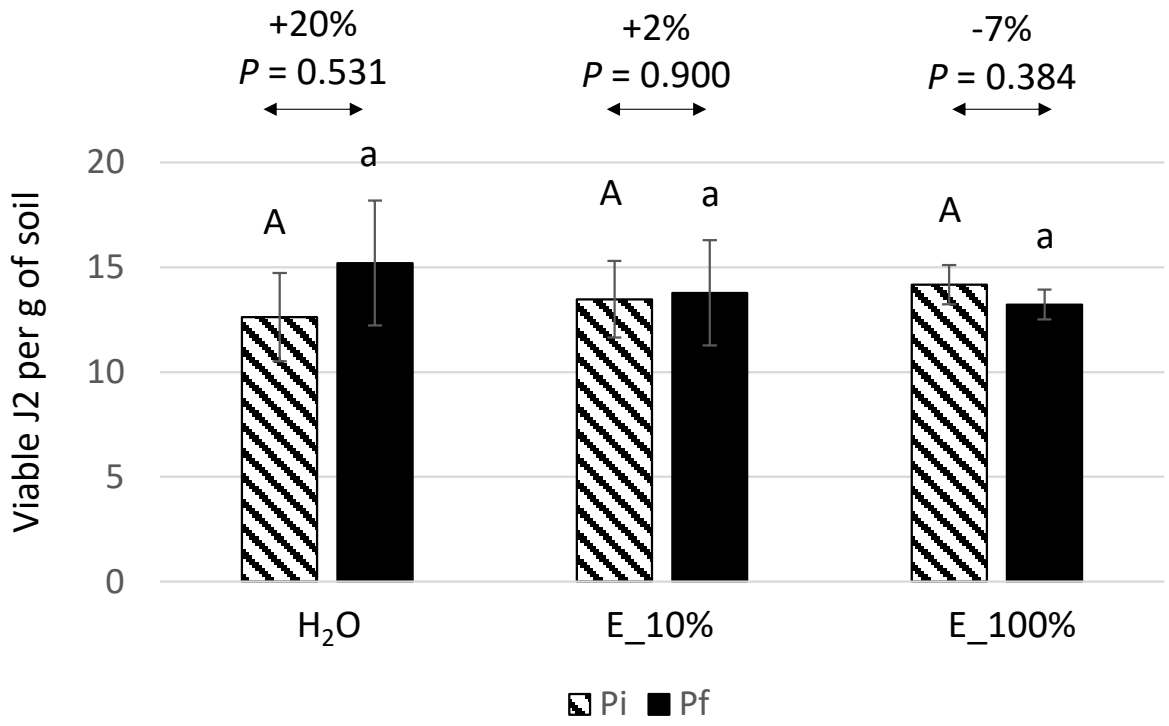
596 **Fig. 5 – Ngala et al**



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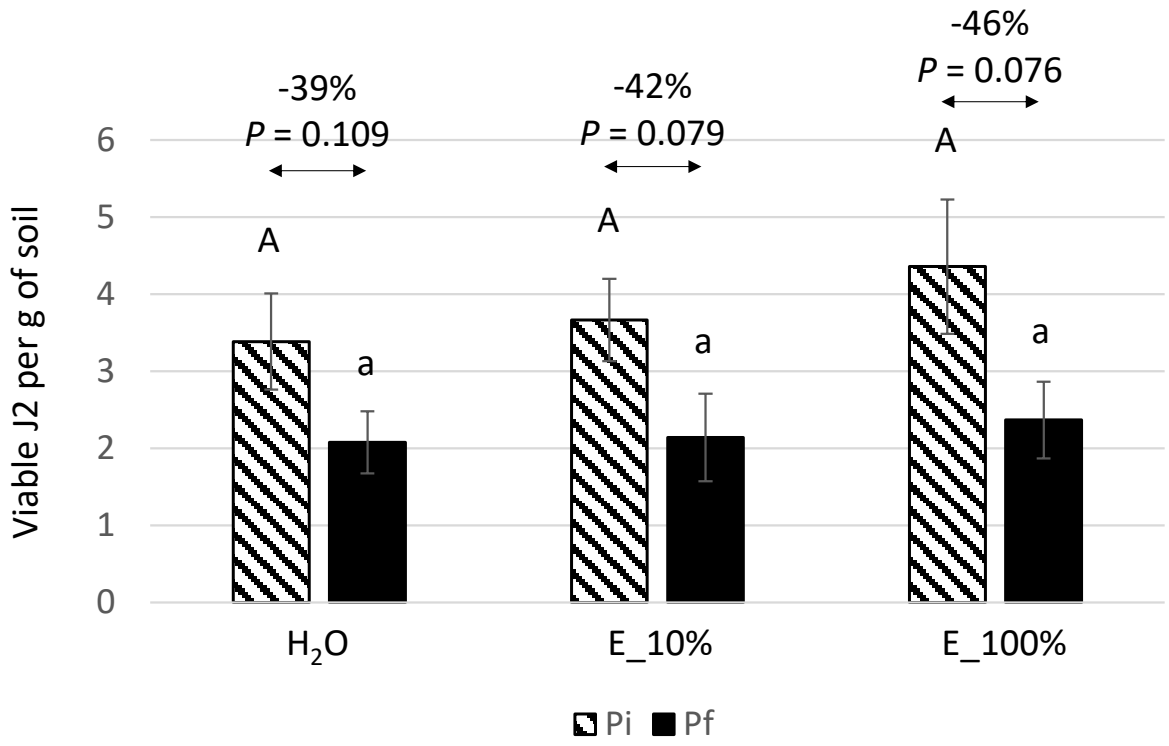
599 **Fig. 6 – Ngala et al**



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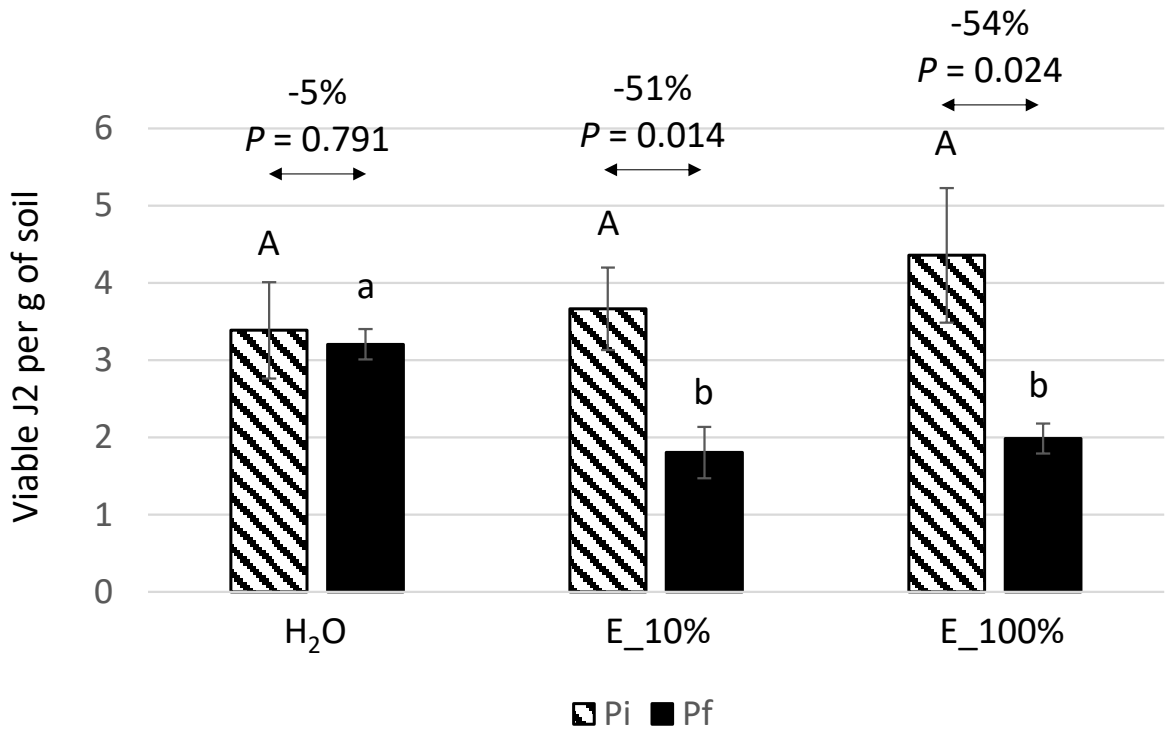
602 **Fig. 7 – Ngala et al**



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