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Aeroponic root leachate (ARL)-induced hatching as a sustainable strategy for the management of *Globodera rostochiensis* in potato (*Solanum tuberosum* L.)

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One of the major challenges in potato farming across the globe is potato cyst nematodes (PCN). Aeroponic root leachate (ARL),collected from aeroponically grown potato plants, was evaluated for its potential to stimulate *Globodera rostochiensis* hatching in the absence of a host plant. In vitro assays showed that ARL collected from 30-day-old potato plants induced the highest number of juveniles (J2s) hatching (369 J2s; 48%), far exceeding that induced by root exudate (RE) (105 J2s; 12.6%). Among the tested dilutions, ARL diluted to 50% was most effective (940 J2s; 74.5%), while controls showed no hatching. Pot assays revealed that ARL diluted to 50% and 75% reduced viable eggs by 28.9% and 27.8%, respectively, compared with minimal reductions in controls (tap water; 4.7% and nutrient solution; 6.2%). Field assays (2018–2021) confirmed strong declines in cyst counts across all treatments, with the greatest reduction observed in T_3 (ARL diluted to 50%). Initial viable egg populations (235–287 per cyst) declined markedly by 2021, with T_3 (ARL diluted to 50%) and T_2 (ARL diluted to 75%) showing 46.5% and 44.1% reductions, compared with controls (13.1% in tap water and 11.2% in nutrient solution). In dose-response assays, ARL triggered higher hatching (284 J2s) than α-chaconine (228 J2s at 100 µg/ml) and α-solanine (186 J2s at 1 µg/ml). Further, ARL-assisted potato farming (ARL-APF) showed lower cultivation costs (643.4 USD/ha), energy inputs (34.5 GJ/ha), carbon inputs (1023.2 kg CE/ha), and GHG emissions (3745.9 CO_2-e kg/ha) over the conventional potato farming (CPF).

Keywords Aeroponic root leachate (ARL), Carbon footprints, *Globodera rostochiensis*, Hatching factors (HFs), Suicide hatching, Viable eggs

Potato cyst nematodes (PCN), namely *Globodera rostochiensis* (golden cyst nematode) and *G. pallida* (white cyst nematode), are the major pests of potato (*Solanum tuberosum* L.). *G. rostochiensis* comprises five pathotypes, Ro1, Ro2, Ro3, Ro4, and Ro5, while *G. pallida* has three pathotypes, Pa1, Pa2, and Pa3[1]. Infested plants exhibit stunted growth, chlorosis, wilting, and reduced tuber production, resulting in significant yield losses[2]. Globally, PCN are responsible for estimated yield losses ranging from 9% to over 30%, depending on the severity of infestation[3,4]. PCN, native to the Andean region of South America, have spread to the major potato-growing regions across the globe through the movement of seed tubers. In India, PCN was first reported from Udhagamandalam, Nilgiris Hills, Tamil Nadu, in 1961 by F.G.W. Jones and later confirmed in Ooctacamund, Tamil Nadu[5]. Since then, their occurrence has been reported in several regions, including the Kodaikanal hills of Tamil Nadu[6], the adjoining

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hills of Karnataka[7], and Pazhathotam, Idukki district, Kerala[8]. Recently, infestations have been documented in various potato-growing areas of the Northwestern Himalayas, notably in Himachal Pradesh, Jammu & Kashmir, and Uttarakhand[9]. Over the years, several nematicides, such as fenamiphos, ethoprophos, cadusafos, fluensulfone, and fosthiazate, have been widely used to prevent infestations of both PCN species. While initially effective, the indiscriminate and prolonged application of these nematicides has led to toxic accumulation in the whole soil-plant-environment continuum[10]. Alternative management practices such as crop rotation, trap cropping, intercropping, and the use of resistant cultivars have been explored[11]. However, the persistence of cysts in soil even without a host complicates these management strategies[12]. Therefore, management of this economically important pest is very difficult and tedious.

PCN are sedentary endoparasites that require a host plant, primarily potato, to complete their life cycle. Once hatched, infective second-stage juveniles (J2s) depend entirely on stored lipid reserves to locate and penetrate host roots. Without a host, these J2s have a limited lifespan in soil, typically surviving only a few weeks as they rapidly deplete lipid reserves, leading to mortality, while unhatched eggs in cysts remain viable for decades. Hatching of cysts is triggered by chemical compounds known as hatching factors (HFs), which are present in potato root exudate (RE). Several HFs have been identified in potato RE, including α-chaconine, α-solanine, solanidine, solasodine, solanoeclepin A, and solanoeclepin B[13–15]. These compounds strongly induce the hatching of *Globodera* spp. in the presence of a host plant, whereas only negligible hatching occurs in water. The highest hatching activity in RE is localized to the root tip region, with peak activity occurring 3 to 4 weeks after plant emergence. Application of such compounds to soil in the absence of a host plant can induce 'suicide hatching', causing J2s to emerge and die without a food source, thereby reducing PCN populations. Consequently, subsequent potato crops can be cultivated with a reduced risk of infestation. Artificial induction of hatching in PCN has been demonstrated in vitro using RE from 3- to 4-week-old potato plants[16]. However, the use of potato as a trap crop to induce hatching followed by ploughing before PCN completes its life cycle [30–35 days after planting (DAP)] is neither economically viable nor practically feasible during the normal potato growing season.

Over the past few decades, aeroponics, a modern soilless agricultural technology, has gained prominence in the global potato seed sector. This innovative technology produces healthy mini-tubers under strict phytosanitary standards while recycling nutrient solution. However, a significant amount of the aeroponic-recycled nutrient solution is discarded as waste. Interestingly, this discarded solution, which cannot escape from closed aeroponic systems, is enriched with root-derived compounds, including potential HFs, and therefore represents a valuable source for PCN management. In India, the aeroponic system was perfected in 2011 and, to date, has been commercialized by 24 firms, each with the capacity to produce up to one million mini-tubers[17]. In this direction, the Indian Council of Agricultural Research-Central Potato Research Institute (ICAR-CPRI), Shimla, Himachal Pradesh, India, has attempted to use this aeroponic-recycled nutrient solution, referred to as aeroponic root leachate (ARL), for PCN management. A patent has also been filed on this innovative approach (Patent Application Number 201811008478, published on 13/09/2019). Based on this concept, the present study assessed the efficacy of ARL in stimulating the hatching of *G. rostochiensis*. In vitro assays were conducted to assess the effect of ARL and RE on nematode hatching. Further, boiling was applied as an experimental stress to evaluate the thermal stability of ARL, and the effect of different storage conditions on its hatching efficiency was also examined. The macro- and microelements in ARL were analyzed. In addition, the efficacy of ARL in stimulating nematode hatching was evaluated under pot and field conditions. The economic and ecological feasibility of ARL-assisted potato farming (ARL-APF) was assessed, and HPLC analysis was performed to quantify α-chaconine and α-solanine in ARL. To our knowledge, this study represents the first global report of ARL as a novel, environmentally friendly strategy for PCN management.

Materials and methods

Collection of ARL

In vitro-grown, virus-free microplants (15 to 21-day-old; approximately 15 cm tall) of the popular Indian potato cultivar Kufri Jyoti were transplanted into 20 mm-diameter holes on the roof of the growth box in the aeroponic unit at the Division of Crop Improvement & Seed Technology, ICAR-CPRI, Shimla. The aeroponic system consists of a light-proof growth chamber, a nutrient solution chamber, filters, spray nozzles, a high-pressure pump, and an electrical unit[17]. Within the enclosed growth chamber, the root system was periodically sprayed with a nutrient solution prepared by dissolving various macro- and microelements (Patent Number 441138). A high-pressure pump forced the nutrient solution as a fine mist through nozzles into growth chambers. The pump operated for 30 s at 5 min intervals, regulated by an electronic timer. This misting process maintained nearly 100% relative humidity (RH), providing optimal conditions for plant growth. Root development began approximately one week after transplanting. A fresh nutrient solution was supplied every 15 days, and the recycled nutrient solution was collected before replacement. This recycled nutrient solution, referred to as aeroponic root leachate (ARL), was filtered twice through Whatman cellulose filters (0.2 µm) and stored at 4 °C for further use.

Collection of RE

The tubers of potato cultivar Kufri Jyoti were grown at a potato farm, ICAR-CPRI, Shimla. RE were collected from 15-, 30-, 45-, 60-, and 90-day-old Kufri Jyoti plants using the root-dipping method[18] at the Division of Plant Protection, ICAR-CPRI, Shimla. Plants were carefully uprooted, washed under tap water to remove soil particles, and rinsed with distilled water. A set of 5 plant roots was dipped in 500 ml of distilled water. The beaker was then sealed with aluminium foil, and kept at 20 ± 2 °C for 24 h. The collected solution was then filtered through 0.2 µm Whatman cellulose filters and stored at 4 °C for subsequent in vitro experiments. All plant-related experiments were conducted in compliance with relevant institutional, national, and international guidelines and legislation.

Nematode culture

The *G. rostochiensis* population was maintained on the PCN-susceptible potato cultivar Kufri Jyoti in the glasshouse at the Division of Plant Protection, ICAR-CPRI, Shimla. Cysts were extracted using the flotation method with a Fenwick can[19]. Healthy cysts were separated from damaged ones under a stereo-zoom microscope (ZEISS SteREO Discovery.V8) and surface-sterilized with 1% sodium hypochlorite for 3 min, followed by 70% ethanol for 2 min, and then rinsed three times with sterile distilled water.

Hatching assays

Effect of ARL and RE on G. rostochiensis hatching

Effect of ARL and RE collected from 15-, 30-, 45-, 60-, and 90-day-old Kufri Jyoti plants were evaluated on the in vitro hatching of *G. rostochiensis* . The nutrient solution from the aeroponic unit and the double-distilled water (DDW) served as controls. ARL and RE were filtered and diluted to 75%, 50%, and 25%. The study consisted of ten treatments: [T_1: ARL (100%); T_2: ARL diluted to 75%; T_3: ARL diluted to 50%; T_4: ARL diluted to 25%; T_5: RE (100%); T_6: RE diluted to 75%; T_7: RE diluted to 50%; T_8: RE diluted to 25%; T_9: DDW; and T_{10}: nutrient solution]. A set of 10 surface-sterilized cysts were placed in 1 ml of respective treatments in a 6-well polypropylene plate and incubated at 20 ± 2 °C under a 16:8 h (dark:light) photoperiod. Plates were sealed with parafilm to prevent evaporation and maintain constant solution volume. Each treatment was performed in ten replicates. The number of hatched J2s was recorded weekly from 7 to 90 days after incubation (DAI) under a stereo-zoom microscope, and the test solutions were replenished after each count. At 90 DAI, cysts were punctured with a pick needle to record the number of unhatched eggs. Hatching percentage was calculated at weekly intervals, and cumulative hatching percentage was determined using the following formula[20].

$$\text{Hatching percentage } (\%) = \frac{\text{Number of hatched juveniles (J2s)}}{\text{Total number of eggs}} \text{ x } 100$$

Effect of boiled ARL on G. rostochiensis hatching

To evaluate the efficacy and stability of ARL on the hatching of *G. rostochiensis*, four treatments were applied: T_1: ARL stored at 4 °C; T_2: ARL boiled at 100 °C for 20 min and stored at 4 °C; T_3: vapours collected during boiling of ARL in the steam distillation apparatus and stored at 4 °C in airtight amber glass bottles; and T_4: DDW as a control. Each treatment involved 20 surface-sterilized cysts with five replicates. The number of hatched J2s from encysted eggs was recorded weekly from 7 DAI up to 7 weeks, and the hatching percentage was calculated as described above.

Effect of storage on the efficacy of ARL on G. rostochiensis hatching

The effect of ARL stored for six months on hatching was evaluated under three conditions: T_1: ARL stored at room temperature (RT) (20 ± 2 °C); T_2: ARL stored at 4 °C; and T_3: ARL autoclaved at 121 °C, 15 psi pressure for 20 min and stored at RT. DDW was kept as a control (T_4). Each treatment had six replicates of 10 surface-sterilized cysts. Hatching of J2s was recorded weekly from 7 DAI up to 7 weeks and the hatching percentage was calculated at the end of the experiment.

Estimation of macro- and microelements in ARL

ARL, a nutrient-rich solution recovered from an aeroponic unit, was analyzed for its macro- and microelement composition. ARL collected from 30-day-old potato plants was processed at the Soil, Water, and Plant Analysis Lab at the Division of Crop Production, ICAR-CPRI, Shimla, following standard methods[21]. Nitrogen (N) was estimated by the Kjeldahl method[22]; phosphorus (P) and sulphur (S) by spectrophotometry[23]; and potassium (K), calcium (Ca), sodium (Na), and lithium (Li) by flame photometry[24]. Other elements, namely magnesium (Mg), zinc (Zn), iron (Fe), copper (Cu), and silicon (Si), were determined using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES)[25].

Effect of ARL on G. rostochiensis hatching under pot conditions

Following the in vitro evaluation of ARL, its efficacy was assessed under controlled glasshouse conditions (20 ± 2 °C) using sterilized sandy loam soil (autoclaved at 121 °C, 15 psi pressure for 20 min on two consecutive days) pre-moistened to 50% field capacity at the Division of Plant Protection, ICAR-CPRI, Shimla. The experiment consisted of six treatments: T_1: ARL (100%); T_2: ARL diluted to 75%; T_3: ARL diluted to 50%; T_4: ARL diluted to 25%; T_5: tap water; and T_6: nutrient solution, where T_5 and T_6 served as controls. Each treatment was replicated three times.

Prior to the experiment, the viability of eggs was assessed using the Meldola's blue staining method[26]. Meldola's blue was procured from Loba Chemie Pvt. Ltd., Mumbai, India. For each treatment, 50 cysts were randomly selected and stained with 0.05% (w/v) Meldola's blue solution. Petri dishes were sealed with parafilm, wrapped in aluminium foil, and incubated at RT for seven days. After incubation, cysts were rinsed with distilled water to remove excess stain, transferred to centrifuge tubes, and gently crushed using a micro-pestle to release the eggs. A 1 ml aliquot from this suspension was observed under a stereo-zoom microscope. Eggs were examined microscopically in six replicates. Unstained eggs were considered viable, whereas stained eggs were classified as non-viable. The results were expressed as the number of viable eggs per cyst, unhatched viable eggs per cyst, and percentage reduction in viable eggs. The percentage of viable egg reduction was calculated as per the following formula:

$$\text{Viable Egg Reduction } (\%) = \frac{(Pi - Pf)}{Pi} X 100$$

Where:

Pi = Initial number of viable eggs (before treatment).

Pf = Final number of unhatched viable eggs (after treatment).

In each pot, measuring 17 cm (D) x 14 cm (H) x 9 cm (B), one sachet containing 20 cysts was placed at 10 cm depth from the topsoil layer. The sachet was made of nylon gauze with a 250 µm mesh size that allowed free diffusion of ARL and migration of hatched J2s into the surrounding soil. Each pot, containing 1.5 kg of double-autoclaved soil, was drenched with 100 ml of the respective treatments at seven-day intervals for one month. The pots were covered with black polythene sheets to maintain soil moisture throughout the experiment. At the end of the experiment, each sachet was carefully cut open, and the cysts were retrieved to count the unhatched viable eggs per cyst (Pf).

Effect of ARL on G. rostochiensis hatching under field conditions

The effect of ARL on *G. rostochiensis* hatching was evaluated under field conditions at the ICAR-CPRI, Kufri farm [31.097858° N, 77.267815° E; ~2,720 m amsl (8,923 feet)] during the kharif season from 2018 to 2021. Prior to the study, the field was planted with the PCN-susceptible cultivar Kufri Jyoti. Following harvest, the field was kept fallow throughout the experiment period. Before treatment application, eighteen plots (1 m × 1 m each) were sampled at a depth of 20–30 cm using 10 soil cores collected in a zigzag pattern. The treatments T_1 to T_6 (as described above) were applied at a rate of 50,000 L/ha using a knapsack sprayer. The experimental site consisted of sandy loam soil, which was rotavated to ensure even distribution within the desired soil profiles following the application of treatment solutions. A randomized block design (RBD) was employed in this study. Plots were covered with black tarpaulins (70 GSM thick) to maintain soil moisture across the treatments. The initial nematode population (Pi) was determined immediately before applying the respective treatments, and the final population (Pf) was assessed 24 weeks after application. Following soil sampling, the fields were covered with black tarpaulins to maintain fallow conditions until the next treatment application in the following year. Pre-treatment soil samples were collected on 25 June 2018, 25 May 2019, and 20 June 2020 for the first, second, and third years, respectively. Post-treatment soil sampling was conducted on 10 December 2018, 8 November 2019, and 4 December 2020.

For each sampling, cyst counts per 100 g of soil were recorded before and after treatment application. To determine the initial viable eggs (Pi) per cyst, 20 cysts were randomly selected three times before the experiment. Similarly, at the end of the study, 20 cysts were randomly collected from each treatment replication to assess the number of unhatched viable eggs per cyst. Based on these observations, the number of viable eggs per cyst, unhatched viable eggs per cyst, and percentage reduction in viable eggs were calculated as described above.

Economic and ecological feasibility of ARL-assisted potato farming (ARL-APF)

The economic and ecological feasibility of ARL-assisted potato farming (ARL-APF) was evaluated per hectare in terms of cost of cultivation (USD/ha), energy inputs (GJ/ha), carbon inputs (kg CE/ha), and greenhouse gas emissions (kg CO2-e/ha) using Cool Farm Tool (CFT) software (https://coolfarm.org/the-tool/), compared to conventional potato farming (CPF) practiced in India.

HPLC analysis of α-chaconine and α-solanine in ARL

HPLC was conducted to quantify α-chaconine and α-solanine in 30-, 45-, and 60-day-old potato plants using standards from Sigma-Aldrich.

Effect of α-chaconine and α-solanine on G. rostochiensis hatching

Working solutions of α-chaconine and α-solanine (100, 10, 1, 0.1, and 0.01 µg/ml) were prepared from a stock solution of 1000 µg/ml. ARL collected from 30-day-old potato plants and DDW were kept as controls along with α-chaconine and α-solanine standards. Each concentration was tested in triplicate, with 5 cysts per replicate. Hatching of J2s was recorded up to 14 DAI of cysts in the respective treatments. At the end of the experiments, the hatching percentage was calculated.

Data curation, analysis, and visualization

To corroborate the relationship between the consequences of different dilutions of ARL and RE, Pearson's correlation analysis was executed and visualized via a correlogram that was constructed employing the "corrgram package" in R Studio. In order to categorize the distinctive patterns in the data, cluster analysis was used. The characteristics of the groups were distinguished based on the variables that were dissimilar. Principal component analysis (PCA), a multivariate analysis that recourses to an orthogonal transformation to transform a set of correlated variables to linearly uncorrelated variables identified as principal components (PC), was resorted to using the Windows-based SPSS version 22. The extracted results of a PCA are presented in terms of component scores, also called factor scores and loadings[27]. Other statistical analysis was done using Statistical Analysis System (SAS v9.3)[28], Pandas (v1.2.0)[29], and Python (v3.9) (https://www.python.org)[30]. In cases where ANOVA was significant, Tukey's honest significant difference (HSD) test was used. At a 5% significance level, correlation analyses and treatment means were compared.

Results

Effect of ARL and RE on *G. rostochiensis* hatching

Among the treatments tested (ARL and RE collected from 15-, 30-, 45-, 60-, and 90-day-old potato plants), a significantly higher number of hatched J2s was recorded only in 30-day-old ARL and RE (Table S1). ARL collected from 30-day-old potato plants induced greater hatching (369 J2s) compared to RE (105 J2s) (Fig. 1). The cumulative hatching percentages were approximately 48% for ARL and 12.6% for RE.

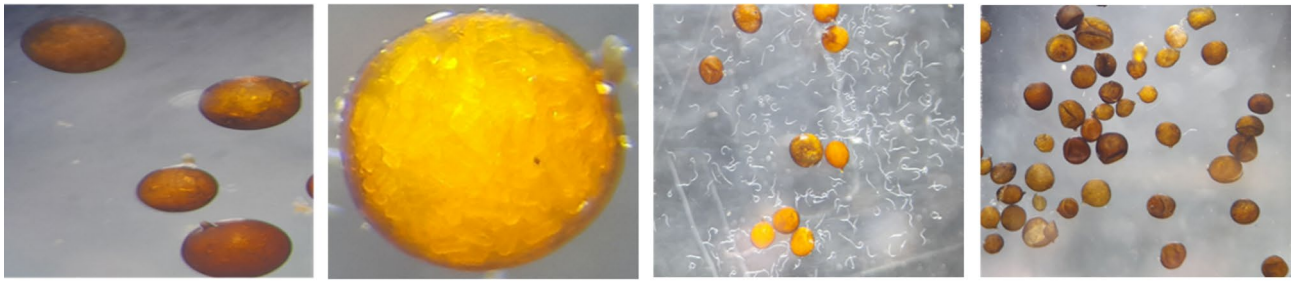

Fig. 1. Hatched juveniles of *Globodera rostochiensis* in aeroponic root leachate (ARL).

Different dilutions of ARL and RE collected from 30-day-old potato plants were also assessed for hatching induction in *G. rostochiensis* eggs. All ARL dilutions showed a significantly higher number of hatched J2s compared to RE 7 DAI. The highest average number of hatched J2s was observed in T_3 (ARL diluted to 50%; 940 J2s), followed by T_2 (ARL diluted to 75%; 719 J2s). In contrast, RE diluted to 25% (T_8) induced a significantly higher number of hatched J2s (340 J2s) compared to the other RE treatments. No hatching was observed in controls (Table S2). Overall, hatching increased during the first week of incubation, peaked in the second week, and then declined across all treatments. The order of hatching was T_3 (ARL diluted to 50%) > T_2 (ARL diluted to 75%) > T_4 (ARL diluted to 25%) > T_1 (100% ARL) > T_8 (RE diluted to 25%) > T_6 (RE diluted to 75%) > T_5 (100% RE) > T_7 (RE diluted to 50%) (Table S3 & S4). The cumulative hatching percentages for different dilutions of ARL and RE were also calculated. Among all the treatments, the highest cumulative hatching percentage was recorded in T_3 (74.5%), followed by T_2 (65.4%), T_4 (62.8%), T_1 (48.9%), and T_8 (44.8%).

A logistic sigmoid model was used to describe the cumulative hatching response of *G. rostochiensis* over time under different treatments with ARL and RE from potato plants of varying ages. The logistic model for nematode hatching was developed, where the response follows an S-shaped (sigmoidal) curve. This pattern includes a slow initial phase, a rapid exponential increase, and a final plateau as the system reaches saturation. The equation used was as follows:

$$\mathrm{Hatch}(t) = \frac{C_{\max}}{1 + \exp\left(-B \cdot (t - A)\right)}$$

Where:

C_{max}: Maximum cumulative hatching (plateau of the curve).

A: Time (in days) required to reach 50% of C_{max}

B: Slope of the curve, representing the speed of hatching

t: Days after incubation (DAI)

This 3-parameter model allowed us to quantify how fast, how much, and when hatching occurs under each studied treatment. The logistic hatching curves in Fig. 2 displayed clear differences in hatching response across the treatments. T_2 (ARL diluted to 75%) and T_3 (ARL diluted to 50%) showed the most vigorous and early hatching responses, reaching high cumulative values before tapering off. T_4 (ARL diluted to 25%) and T_8 (RE diluted to 25%) showed moderate hatching with a slower slope and lower overall cumulative hatch compared to T_2 and T_3. RE treatments (T_5 to T_7) demonstrated minimal to low cumulative hatch values, with T_7 (RE diluted to 50%) and T_5 (100% RE) showing especially poor response. T_9 (DDW) and T_{10} (nutrient solution) showed virtually no hatching, confirming that observed hatching in other treatments was specifically induced by specific cues present in ARL and RE. The logistic parameter estimates [maximum cumulative hatch (C_{max}), inflection point (A), and slope parameter (B)] along with model fit metrics root mean square error (RMSE) and pseudo R^2 across treatments is shown in Table S5.

Effect of boiled ARL on *G. rostochiensis* hatching

A significantly higher number of hatched J2s was recorded in T_1 (ARL; 1234 J2s), followed by T_2 (boiled ARL; 236 J2s), T_3 (ARL vapour; 139 J2s), and T_4 (DDW; 30 J2s). These results indicate that boiling substantially reduces the stability and hatching-stimulatory activity of ARL. Across all treatments, hatching continued for up to 7 weeks of cyst incubation, with the highest numbers observed during the first three weeks, followed by a gradual decline (Fig. 3a). The cumulative hatching percentage was highest in T_1 (71.2%), followed by T_2 (22.8%), T_3 (13.5%), and T_4 (2.2%).

Effect of storage on the efficacy of ARL on *G. rostochiensis* hatching

In the ARL storability study, hatching of J2s was recorded across all treatments. A significantly higher number of hatched J2s was observed in T_2 (ARL stored at 4 °C; 506 J2s), followed by T_3 (autoclaved ARL; 439 J2s), T_1 (ARL stored at RT; 224 J2s), and T_4 (DDW; 19 J2s) (Fig. 3b). Cumulative hatching percentages were 54.6% in T_2, 26.4% in T_3, 23.5% in T_1, and 1.9% in T_4.

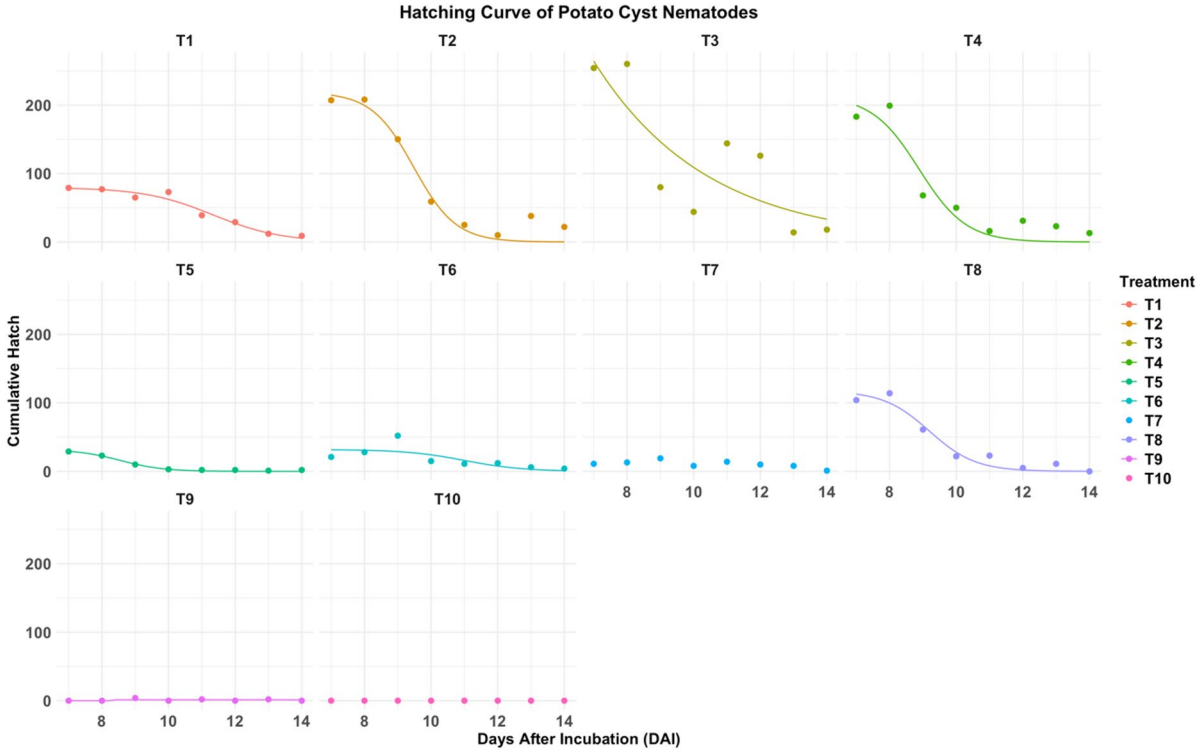

Fig. 2. Hatching curve of *Globodera rostochiensis* over time among different treatments.

Estimation of macro- and microelements in ARL

To assess potential effects on soil fertility, the concentration of macro- and microelements in ARL was measured in parts per million (ppm). N was undetectable, and other elements were recorded in amounts lower than typical plant requirements. K was the only element detected at a relatively higher level (227 ppm), which may be attributed to reduced root growth after 30 days (Fig. 4 & Table S6). These findings suggest that ARL can be safely applied under field conditions without adversely affecting soil fertility.

Effect of ARL on *G. rostochiensis* hatching under pot conditions

Following in vitro evaluation, the efficacy of ARL was further assessed under glasshouse conditions. Before treatment, 20 cysts were randomly selected from each treatment in triplicate to determine the initial viable eggs (Pi) per cyst, which ranged from 327 to 348 eggs. Among the treatments, a significant reduction in viable eggs per cyst was recorded in T_3 (ARL was diluted to 50%; 28.9%) and T_2 (ARL diluted to 75%; 27.8%). In contrast, the controls showed only minor reductions: T_5 (tap water; 4.7%) and T_6 (nutrient solution; 6.2%) (Fig. 5).

Effect of ARL on *G. rostochiensis* hatching under field conditions

In field experiments conducted from 2018 to 2021, the initial cyst population (Pi) ranged from 486 to 514 per 100 g of soil. Each year, the plots were treated with the respective treatments and covered with a black polythene sheet. Across all ARL treatments, cyst populations were significantly reduced compared to the controls, with the greatest reduction of 66.06% in T_3 (ARL diluted to 50%), followed by 60.59% in T_1 [ARL (100%)] (Fig. 6).

The initial viable egg population (Pi) in 2018 ranged from 235 to 287 per cyst, while the final unhatched viable eggs (Pf) in 2021 showed significant reductions in T_3 (ARL diluted to 50%) with a 46.5% reduction and T_2 (ARL diluted to 75%) with a 44.1% reduction. Controls showed only minor reductions: tap water (13.1%) and nutrient solution (11.2%) (Fig. 7).

Economic and ecological feasibility of ARL-assisted potato farming (ARL-APF)

The economic and ecological feasibility of ARL-assisted potato farming (ARL-APF) was evaluated in comparison with conventional potato farming (CPF) using Cool Farm Tool (CFT) software (https://coolfarm.org/the-tool/). The results showed that ARL-APF reduced cultivation costs (643.4 USD/ha), energy inputs (34.5 GJ/ha), carbon inputs (1023.2 kg CE/ha), and GHG emissions (3745.9 CO_2-e kg/ha) over the CPF, which had respective values of 654.2 USD/ha, 35.97 GJ/ha, 1047.1 kg CE/ha, and 3788.9 CO_2-e kg/ha (Fig. 8).

HPLC analysis of α-chaconine and α-solanine in ARL

HPLC analysis was conducted to detect α-chaconine and α-solanine in ARL collected from 30-, 45-, and 60-day-old potato plants. 16.2 µg/100 ml of α-chaconine and 7.60 µg/100 ml of α-solanine were observed in ARL from 30-day-old potato plants, with J2s hatching observed only at this stage (Fig. 9). A one-way ANOVA revealed highly significant differences ($p < 0.001$) in the concentrations of both α-chaconine and α-solanine and across the

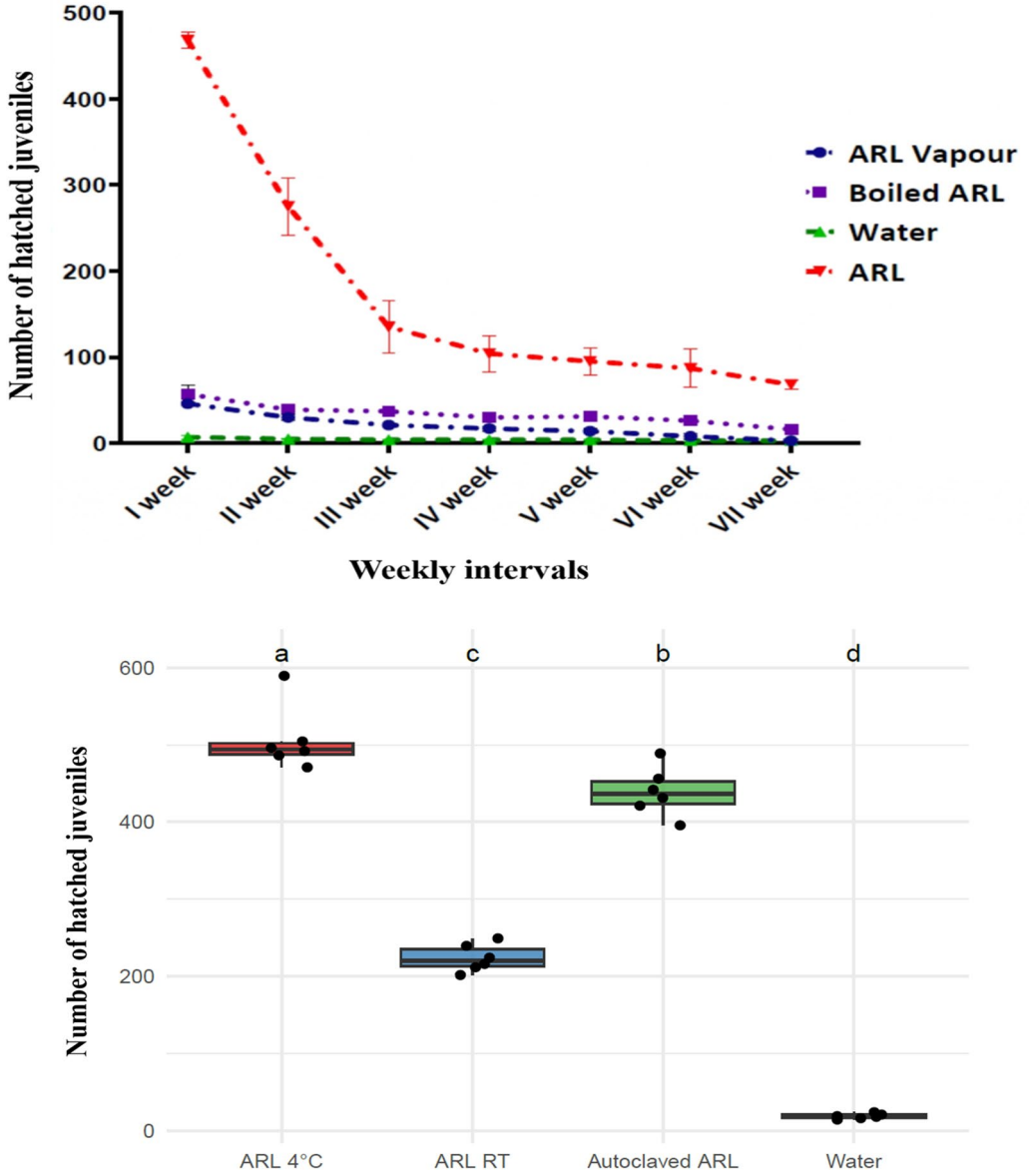

Fig. 3. **a**) Temporal pattern of *Globodera rostochiensis* hatching under different aeroponic root leachate (ARL) treatments over a 7-week incubation period; **b**) Cumulative hatching response across treatments. Different letters above bars indicate statistically significant differences (LSD, $p \leq 0.05$).

three sampling intervals (30, 45, and 60 days). For α-chaconine, the F-value was 623 with a p-value of 1.1×10^{-7}, while for α-solanine, the F-value was 198.3 with a p-value of 3.31×10^{-6}.Post-hoc Tukey's HSD tests further confirmed that glycoalkaloid concentrations were significantly highest at 30 days, followed by a decline at 45 days, and lowest at 60 days, with all time points forming distinct statistical groups (30d: *a*, 45d: *b*, 60d: *c*) for both compounds. These results validate that the ARL collected from 30-day-old potato plants is chemically the most potent and align with the observed nematicidal effects.

Effect of α-chaconine and α-solanine on *G. rostochiensis* hatching

Different concentrations of α-chaconine and α-solanine were tested for their effect on J2s hatching. The highest number of hatched J2s (228) was observed in the 100 µg/ml of α-chaconine, while for α-solanine, the highest number of hatched J2s was 186 at the 1 µg/ml (Fig. 10a & b). However, ARL showed a significantly higher hatching rate (284 J2s), indicating the presence of other unidentified compounds responsible for the hatching.

Mixed-model interaction study

A linear mixed-effects model was applied to evaluate treatment efficacy across years (2018–2021) for both cyst reduction and viable egg reduction in field study. In the cyst reduction model, where treatment was treated as a fixed effect and year as a random effect, the model fit was acceptable (REML = 521.2), with residual and year-level standard deviations of 10.95 and 3.46, respectively. Treatments T_5 and T_6 showed statistically significant

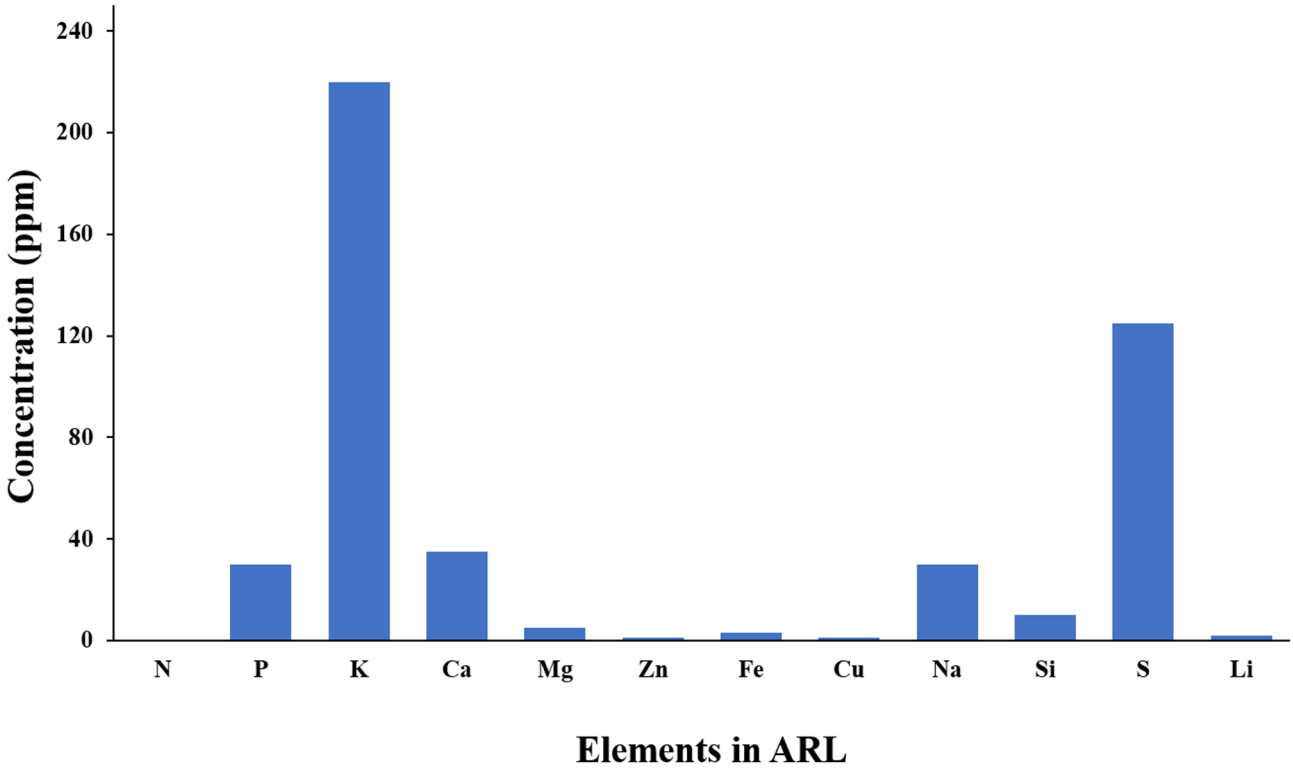

Fig. 4. Status of macro- and microelements in aeroponic root leachate (ARL).

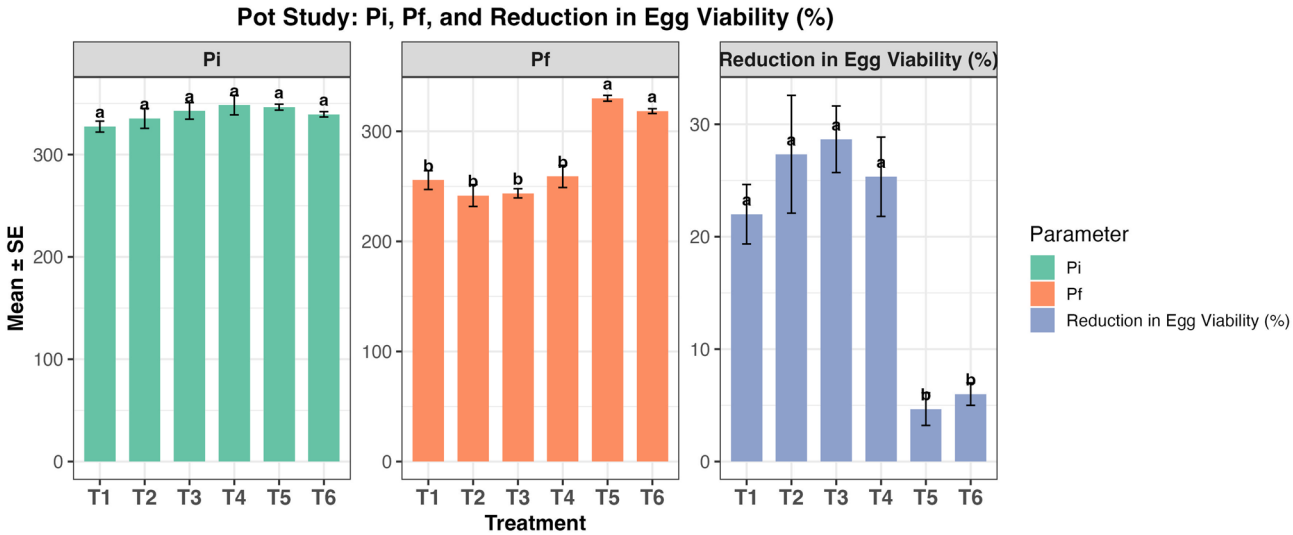

Fig. 5. Treatment-wise comparison of initial viable eggs (Pi) per cyst, final viable eggs (Pf) per cyst, and reduction in egg viability (%) under pot conditions.

reductions in cyst counts compared to the control (T_1), with estimates of -12.38 ($p = 0.007$) and -12.95 ($p = 0.005$), respectively. Although T_3 exhibited a numerical improvement (+2.72 units), it was not statistically significant ($p = 0.545$). Similarly, in the viable egg reduction model (REML = 378.9), the year contributed moderate variance (SD = 1.57), and the residual SD was 3.69. T_5 and T_6 again showed highly significant reductions (-9.26 and -9.79; $p < 0.001$), whereas T_3's reduction (+1.74) was not statistically significant ($p = 0.254$). These results confirm that treatments like T_3 showed strong biological trends, with cyst and viable egg reductions nearing 66% and 46.5%, respectively; however, T_5 and T_6 emerged as the only treatments with consistently significant reductions across years, validating the use of a mixed-model to account for year-wise variability.

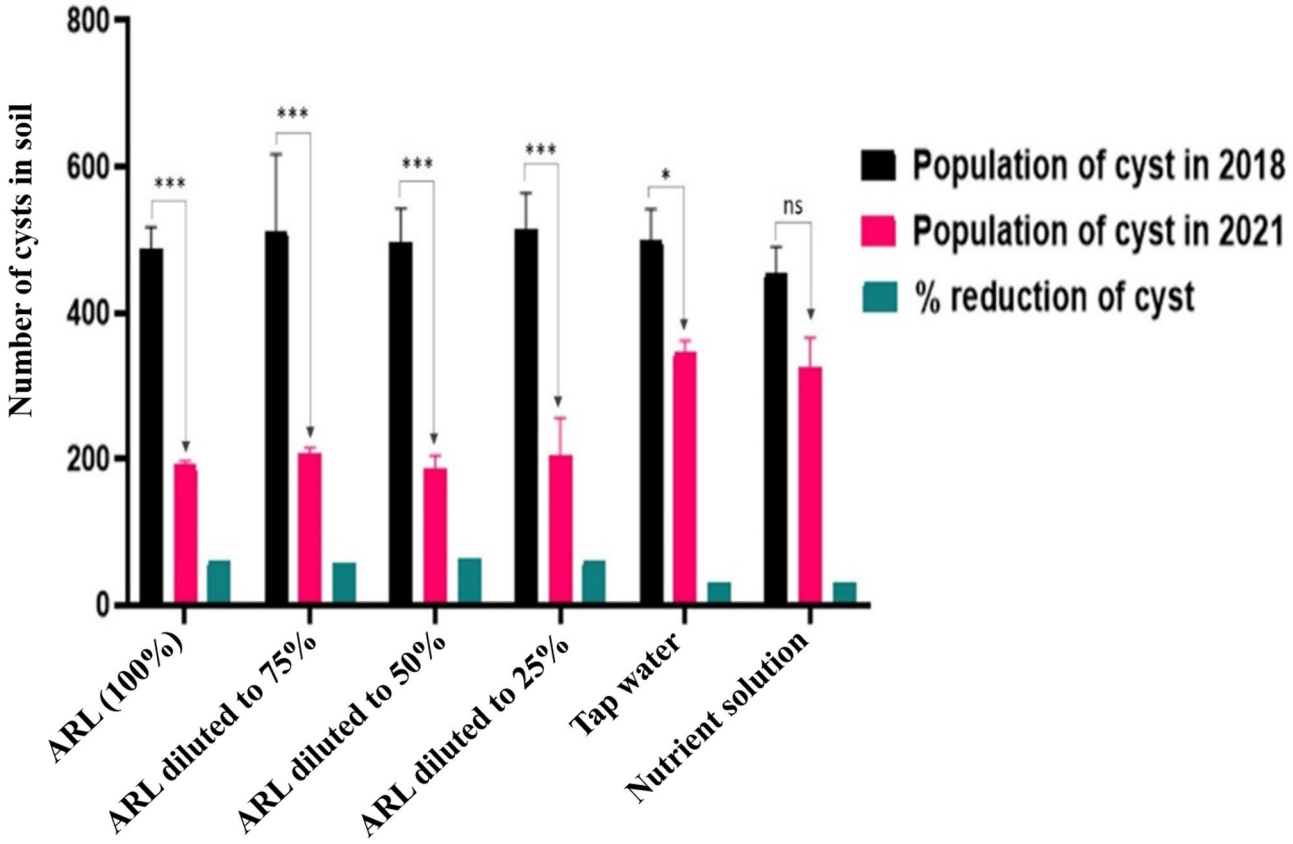

Fig. 6. Effect of different aeroponic root leachate (ARL) dilutions on cyst count of *Globodera rostochiensis* in soil (2018 to 2021) (*significant at $p < 0.05$, *** significant at $p < 0.0001$, and ns: non-significant).

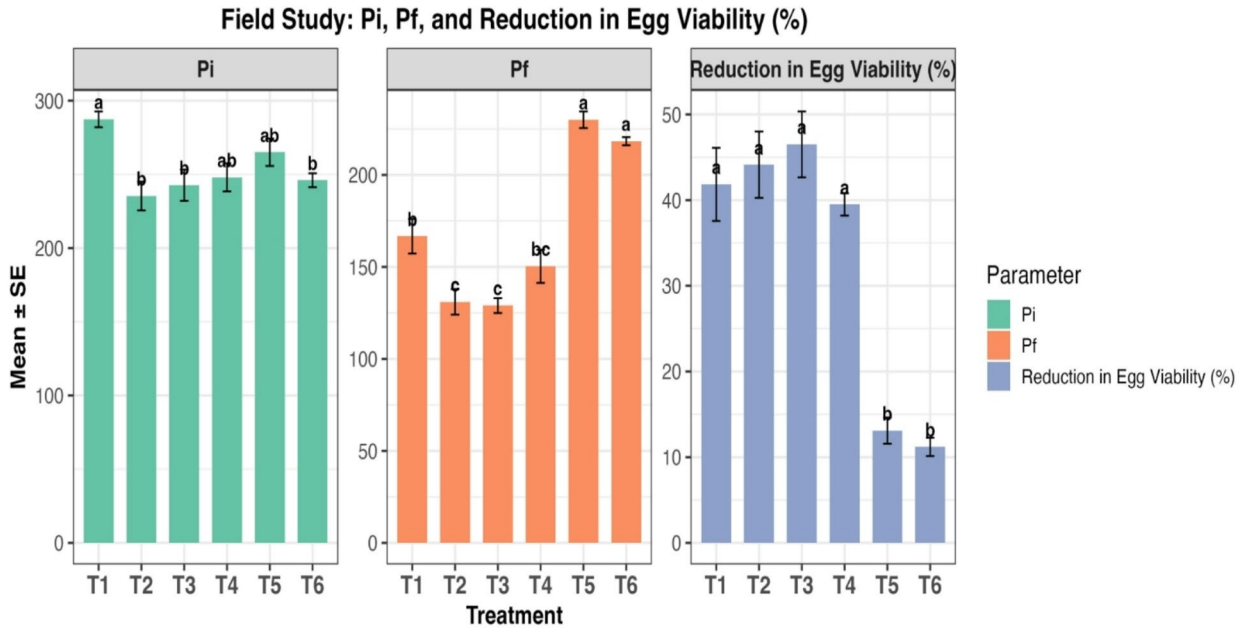

Fig. 7. Treatment-wise comparison of initial viable eggs (Pi) per cyst, final viable eggs (Pf) per cyst, and reduction in egg viability (%) under field conditions.

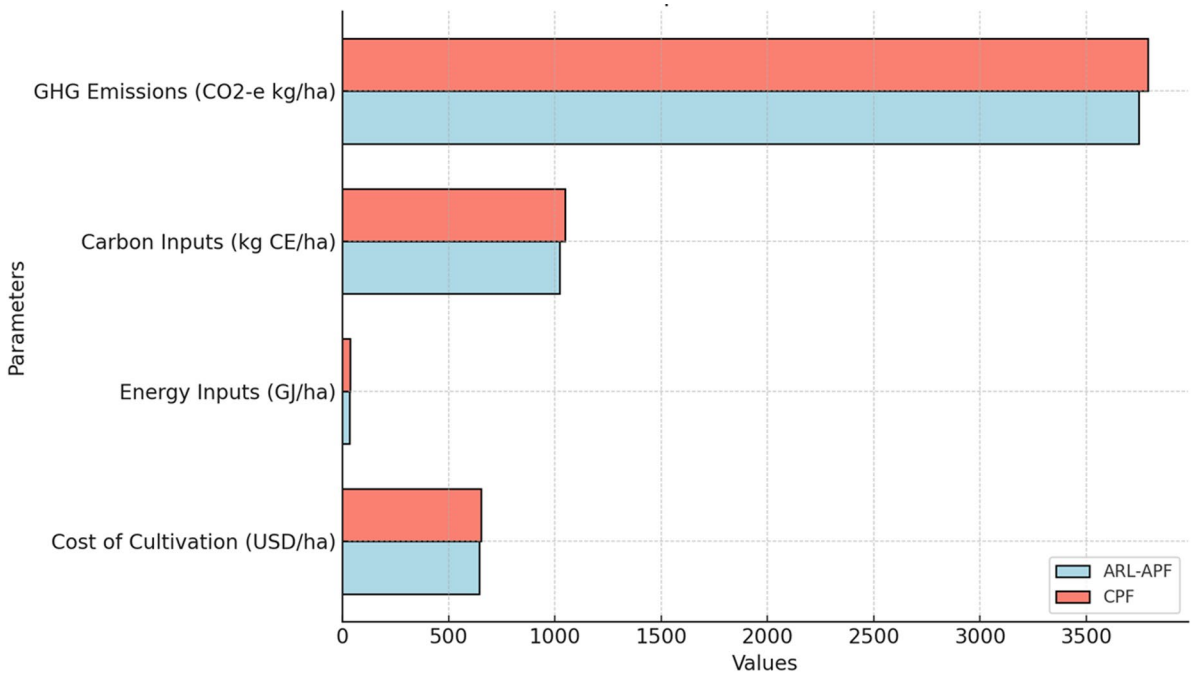

Fig. 8. Economic and ecological impact of ARL-assisted potato farming (ARL-APF) in reducing production costs, energy investment, carbon inputs, and GHG emissions in PCN-infested hill ecosystems.

The results from the mixed-model analysis, visualized through the Fig. 11a & b, clearly illustrate the differential performance of treatments across years. In Fig. 11a, which displays the interaction effects on the percentage reduction in viable eggs per cyst, treatment T_3 (ARL diluted to 50%) consistently outperformed all other treatments across the years 2018 to 2021. Its steep decline in viable egg count demonstrates superior efficacy, followed by T_2 (ARL diluted to 75%), while the control treatments (T_5 and T_6) showed only minor reductions.

The Fig. 11b, focused on the percentage reduction in cyst counts, further supports this trend again, T_3 showed the most substantial and consistent reduction across all four years, confirming its robust suppressive effect on nematode buildup. The inclusion of 95% confidence intervals adds strength to these observations, showing narrow variability and reinforcing the statistical confidence in treatment efficacy. These patterns reflect significant treatment × year interaction effects, justifying the mixed -model framework that treated year as a random effect and treatment as fixed. The visual clarity in both plots enhances interpretation of the model output and substantiates the conclusion that ARL diluted to 50% (T_3) is the most promising treatment for sustainable cyst nematode management.

Discussion

To date, ARL has not been recognized as a bio-based substance capable of inducing hatching in *G. rostochiensis*. This study is the first to systematically demonstrate its potential application in environmentally friendly management of this pest. Globally, strict quarantine regulations have been enforced to limit the spread of PCN, owing to their destructive impact and the difficulty of eradication once established in the field[31]. A four-week-old potato plant releases specific HFs that stimulate PCN hatching. This is critical because newly hatched J2s have a limited period to locate, penetrate, and establish within host roots[32]. Consequently, the nematode's survival depends on these host-derived chemical cues.

Effect of ARL and RE on *G. rostochiensis* hatching

The hatching of J2s of *G. rostochiensis* was recorded only in ARL and RE collected from 30-day-old potato plants across all treatments. This finding agrees with[33], who reported minimal hatching in RE during the first two weeks of planting due to the presence of hatching inhibitors (HIs). Similarly[34], observed maximum hatching of *G. rostochiensis* in potato root diffusate (PRD) collected three weeks after plant emergence, followed by a decline thereafter. Additionally, hatching was positively correlated with root weight during the initial three-week period. It could be due to reproductive development in plants, which may change the photosynthetic partitioning. Our in vitro assays identified ARL from 30-day-old potato plants as the optimal collection stage. Further, various dilutions of ARL and RE from 30-day-old potato plants were evaluated for inducing the hatching of *G. rostochiensis*. All ARL dilutions resulted in significantly higher hatching of J2s than RE, with maximum hatching in T_3 (ARL diluted to 50%; 940 J2s), followed by T_2 (ARL diluted to 75%; 719 J2s). Among RE treatments, only T_8 (RE diluted to 25%) induced a significantly higher hatching (340 J2s). It was noted that even ARL diluted to 25% induced significantly higher hatching than equivalent RE dilution. Although nematode hatching has previously been reported from RE[18,32,35,36], no prior studies have documented hatching in ARL. The stronger

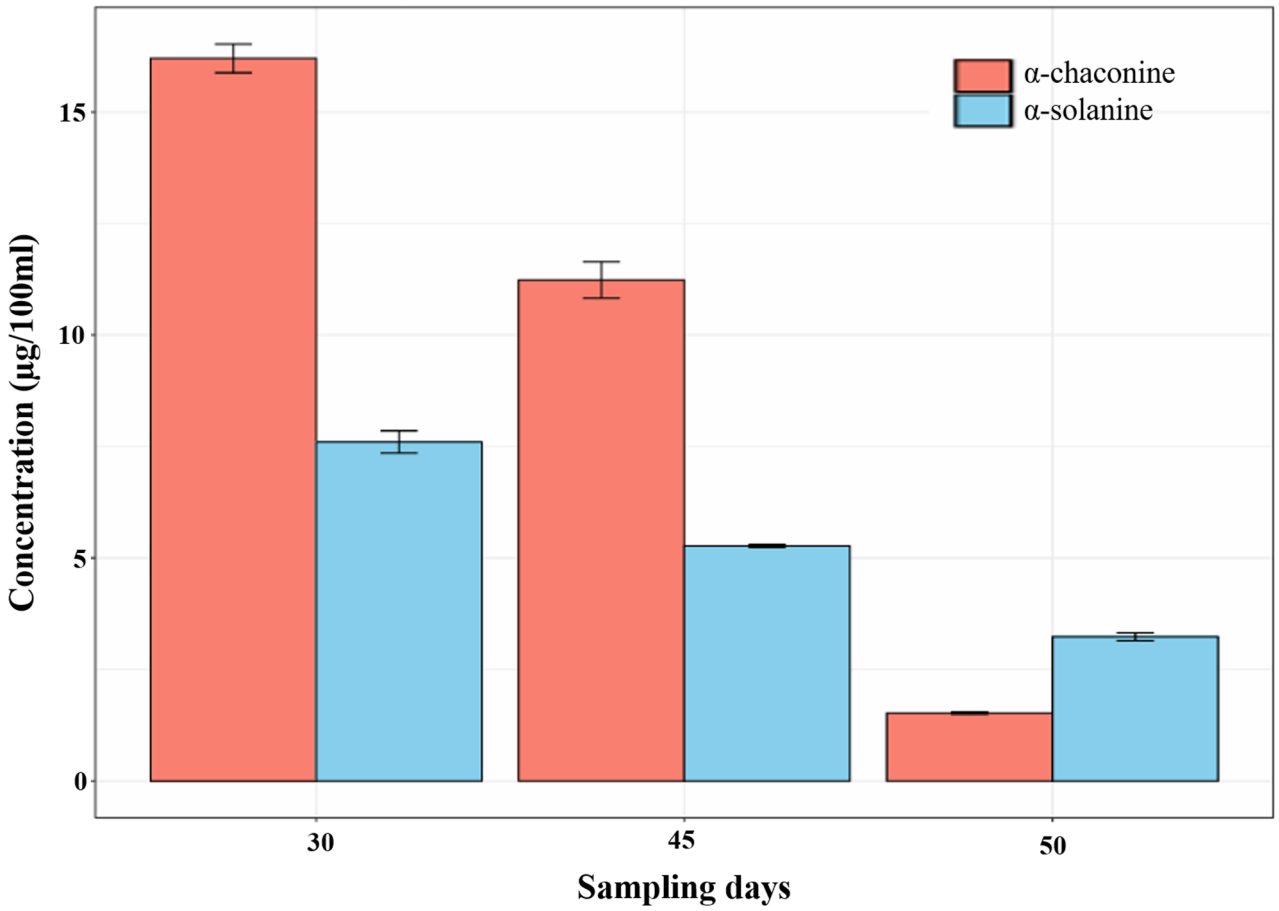

Fig. 9. Concentrations of α-chaconine and α-solanine in the aeroponic root leachate (ARL) at different sampling days.

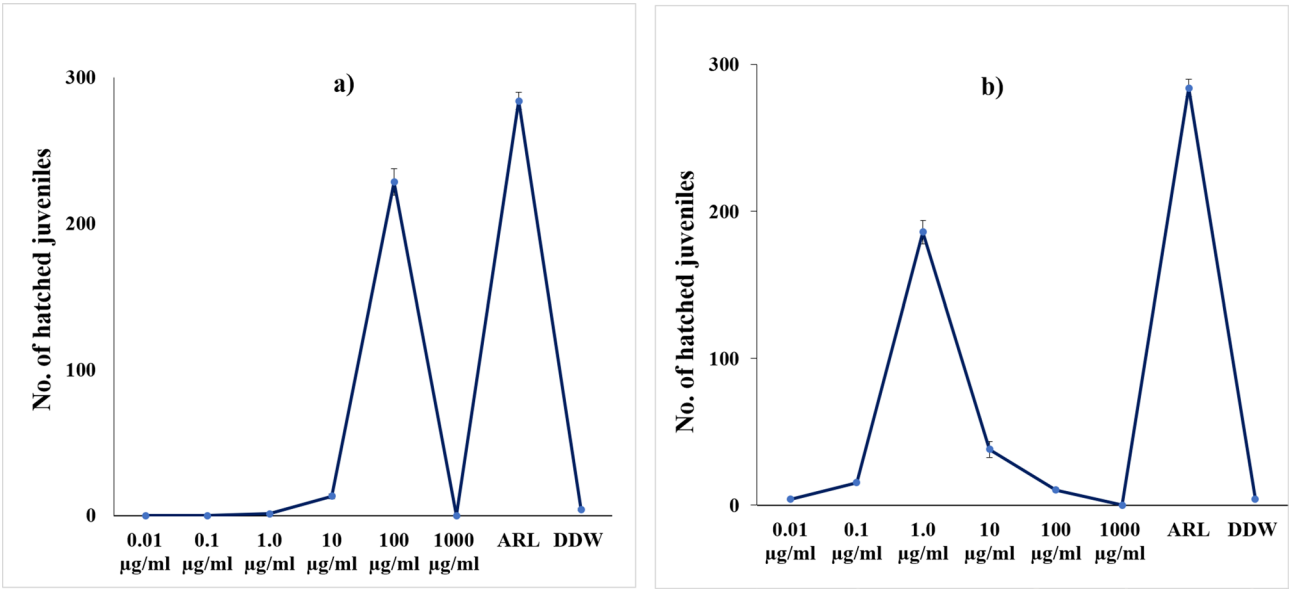

Fig. 10. **a**) Number of hatched juveniles (J2s) of *Globodera rostochiensis* in different concentrations of α-chaconine; **b**) Number of hatched juveniles (J2s) of *Globodera rostochiensis* in different concentrations of α-solanine.

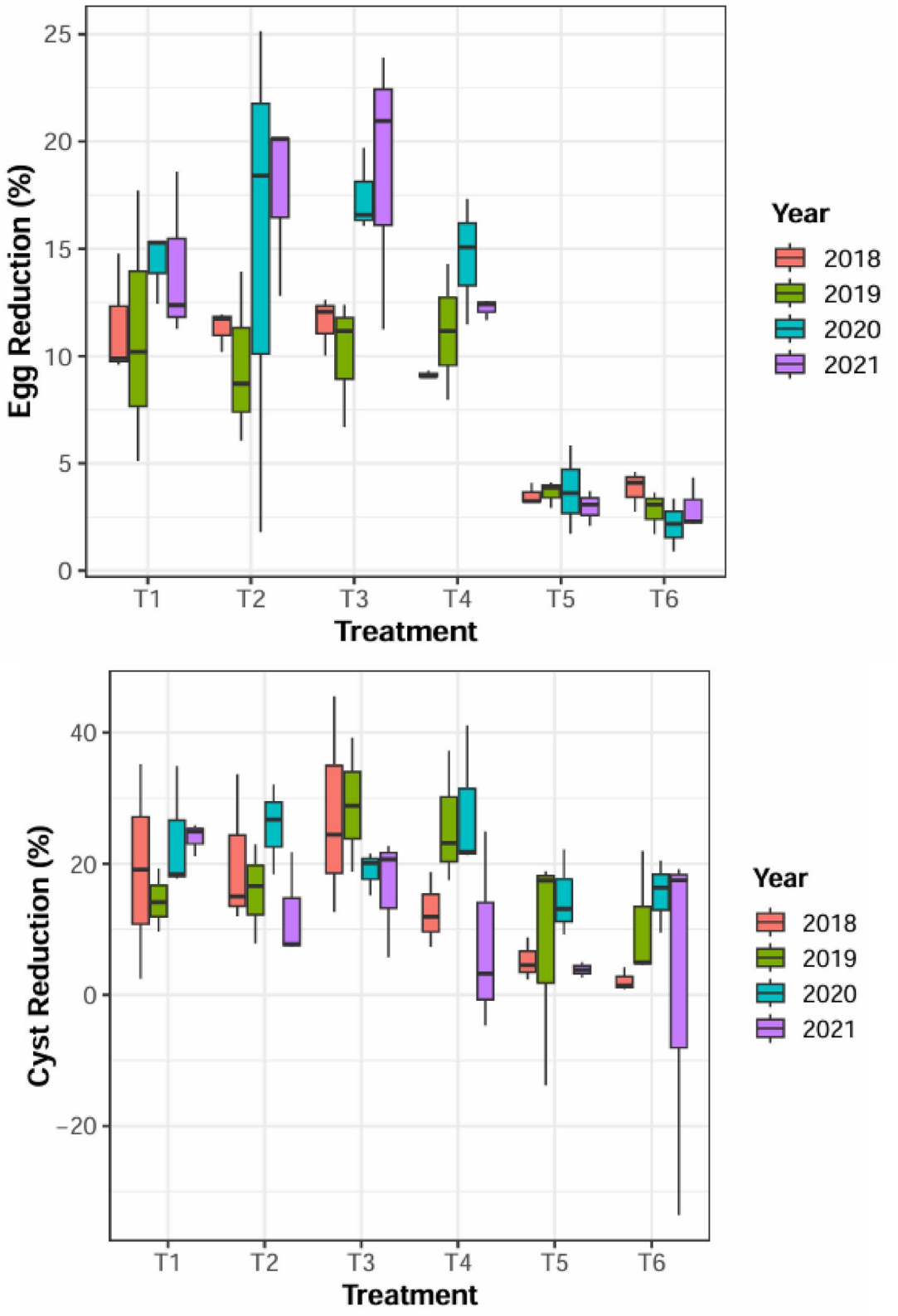

Fig. 11. **a**) Interaction effects of treatment efficacy across years (2018–2021) on viable egg reduction (%); **b**) Interaction effects of treatment efficacy across years (2018–2021) on cyst reduction (%).

nematode hatching observed with diluted ARL may be due to the fact that, at optimal or lower dilutions, HFs may bind specifically to egg receptors and effectively trigger the hatching. In contrast, undiluted ARL may cause receptor overstimulation, feedback inhibition, or dominance of inhibitory compounds that suppress the hatching response.

Strikingly, ARL induced nearly threefold more J2 hatchings than RE, highlighting its strong stimulatory potential. Aeroponics promotes plant growth by suspending roots in air and periodically misting them with a sterile nutrient solution. This controlled environment increases plant biomass, root development, and exudate production while enabling the collection of root leachates with minimal microbial degradation and soil sorption. In contrast, RE in soil are rapidly metabolized by the rhizosphere microbiome and adsorbed onto mineral and organic surfaces, altering the composition and potency of active HFs. Therefore, ARL likely retains higher levels of intact, bioactive exudate fractions and demonstrates stronger stimulation of nematode hatching compared to soil-derived RE.

Effect of boiled ARL on *G. rostochiensis* hatching

Boiling reduced ARL's efficacy for inducing hatching, with activity ranked as ARL (T_1) > boiled ARL (T_2) > ARL vapours (T_3) > DDW (T_4). Both boiled ARL and ARL vapours induced significantly fewer hatched J2s. This likely results from thermal degradation or denaturation of key HFs. Reduced or delayed hatching indicates that the boiling slows down the activity of hatching-inducing compounds compared to the unboiled ARL. The DDW control serves as a baseline for spontaneous hatching in the absence of HFs. Abiotic factors, including temperature extremes, drought, high salinity, flooding, and nutrient starvation, affect the root exudation process in plants[37]. Our study is in corroboration with[38,39], who reported that thermal degradation of different bioactive compounds depends on temperature.

Effect of storage on the efficiency of ARL on *G. rostochiensis* hatching

The storability study revealed distinct patterns on the efficacy of ARL in hatching *G. rostochiensis*. ARL stored at 4 °C (T_2) exhibited the highest hatching response (506 J2s; 54.6%), indicating that lower temperature storage preserves hatching activity. In contrast, ARL stored at RT (T_1) and autoclaved ARL (T_3) showed reduced hatching (224 J2s; 23.5% and 439 J2s; 26.4%, respectively), suggesting that prolonged storage at RT and exposure to autoclaving accelerated degradation of HFs, resulting in a decline in hatching over six months. The minimal hatching observed in DDW (T_4; 19 J2s: 1.9%) confirmed that the effects were attributable to ARL-derived compounds. Sudden fluctuations in temperature and heat stress accelerate the breakdown of these compounds, diminishing hatching efficiency. These results align with[40], who found that lower growth temperatures (10 °C-day and 5 °C-night) increased the levels of bioactive compounds, antioxidant activities, and antioxidant enzymes in both wheat and barley.

Effect of ARL on *G. rostochiensis* hatching under pot conditions

Following the in vitro studies, the efficacy of ARL was subsequently evaluated under pot conditions. A significant reduction in viable eggs per cyst was recorded in T_3 (28.9%), which was statistically at par with T_2 (27.8%), followed by T_4 (25.6%) and T_1 (21.8%). In contrast, reductions in viable eggs per cyst were minimal in the tap water (T_5; 4.7%) and nutrient solution (T_6; 6.2%). The ARL was collected at 30 DAP from aeroponically grown potatoes, ensuring samples were free from soil particles and microbiota that could degrade the HFs. The higher number of viable eggs in controls likely reflects the absence of HFs. These findings demonstrate that ARL alone, under favourable soil conditions (14–20 °C temperature and 60–80% moisture), was sufficient to induce hatching in PCN even without a host. Our results align with[32], who reported *Heterodera carotae* hatching was similar in RE diluted to 25% and 10%, indicating that even low dilutions can still be effective. This may be due to the fact that RE are rapidly adsorbed onto soil particles and undergo microbial degradation. Additionally, the exudation from the soil microbiota also affects the plant exudation profile. After the pot study, more empty eggs were observed in ARL-treated pots, supporting its role as a strong 'hatching inducer'.

Effect of ARL on *G. rostochiensis* hatching under field conditions

From 2018 to 2021, ARL treatments consistently resulted in significant reductions in cyst counts compared to the controls. The soil was saturated to a depth of 30 cm and thoroughly mixed with ARL to facilitate egg hatching. The maximum significant reduction in cyst counts and viable eggs was observed in T_3 (ARL diluted to 50%)[41]. found that applying HFs exogenously before planting reduced the *G. rostochiensis* population by 50% due to an increase in egg mortality. Similarly[42], observed hatching of *G. rostochiensis* and *G. pallida* at a planting depth of 20 cm. In the present study, we also found some reductions in the cyst counts in both controls. It may be due to the spontaneous hatching of eggs in some cysts. Our study aligns with[36], who observed that 25.95% of *G. pallida* eggs hatched spontaneously without a host, depending on environmental conditions. Interestingly, unlike in vitro assays (where hatching was negligible in controls), soil seemed to intrinsically promote some hatching, possibly through microbial enzymatic effects on eggshell[43,44]. The use of sandy loam soils in the field likely contributed to the satisfactory results. [45] found that the 'suicide hatching' technique was more effective in sandy soils than in clay or peat soils. Trap cropping is also being utilized to catch cyst nematodes, where hatched J2s are attracted to host roots, but failure to uproot the plant in time can result in new cyst formation[31]. By contrast, ARL application provides a controlled, host-free method for inducing 'suicide hatching'. Furthermore, ARL contains beneficial macro- and microelements and phytochemicals, is low-cost, and is environmentally friendly, potentially enhancing potato growth and reducing nematode population.

Economic and ecological feasibility of ARL-assisted potato farming (ARL-APF)

The economic and ecological feasibility of ARL-APF was compared with CPF using CFT software. The analysis showed that ARL-APF reduced overall cultivation costs, energy inputs, carbon inputs, and GHG emissions over the CPF. Since ARL is a plant-derived solution, its use reduces the high energy demand, carbon footprint, and GHG emission. [46] investigated the impact of integrated crop management (ICM) on potato productivity, profitability, energy use, and carbon footprints in the northwestern Himalayas. They reported that ICM practices significantly improved potato yields, economic returns, and resource-use efficiency while reducing environmental impacts such as energy consumption, carbon inputs, and GHG emissions compared to CPF.

HPLC analysis of α-chaconine and α-solanine in ARL

In the ARL sample collected from 30-day-old potato plants, we detected 16.2 µg/100 ml of α-chaconine and 7.60 µg/100 ml of α-solanine through HPLC analysis. Steroidal glycoalkaloids (SGAs) are predominantly found in the Solanaceae family. Among various SGAs, α-chaconine and α-solanine account for 95% of total glycoalkaloid in potato. Structurally, both compounds have a steroidal alkaloid part known as aglycone solanidine, to which a trisaccharide is attached[47].

Effect of α-chaconine and α-solanine on *G. rostochiensis* hatching

Different concentrations of α-chaconine and α-solanine were evaluated for *G. rostochiensis* hatching. The highest number of hatched J2s (228 J2s) for α-chaconine was recorded at 100 µg/ml, while α-solanine showed its maximum hatching response (186 J2s) at 1 µg/ml. It was found that the α-chaconine and α-solanine elicited similar hatching response patterns, which varied depending on the concentration, indicating that the type of sugar linked to the aglycone may influence the stimulation response of J2s. Our results align with[48], who reported maximum hatching of *G. rostochiensis* at 10^{-3} M concentrations of both α-chaconine and α-solanine. Our findings also corroborate with[13,33,49], who have identified α-chaconine and α-solanine as potential HFs.

Conclusion

Potato ARL triggers 'suicide hatching' in *G. rostochiensis*, a process that disrupts the nematode's life cycle and reduces soil population densities. This strategy, consequently lowers dependence on synthetic nematicides and mitigates their adverse ecological effects on soil health. Future research should focus on identifying and synthesizing the active hatching compounds and on developing optimized, stable formulations for practical field application.

Data availability

All the data generated in this study are included in the manuscript. Additional data are provided in the supplementary file.

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Author contributions

AB and TB conceived the idea and designed the research framework. SS, AKS, SKC, and BrS provided resources and supervision. AB, BD, DD, and KCN performed the laboratory, pot, and field experiments and curated the data. BS, PC, and IB conducted the data analysis. AB, TB, and BD prepared the original manuscript. AB, BD, PC, AKC, PHM, and SK critically reviewed the final manuscript. All authors have approved the submitted version of the manuscript.

Declarations

Competing interests
The authors declare no competing interests.

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