

RESEARCH/INVESTIGACIÓN

DISTRIBUTION OF CEREAL CYST NEMATODES IN RICE FIELDS OF PUNJAB, PAKISTAN AND EVALUATION OF NEMATICIDAL POTENTIAL OF RHIZOBACTERIA TO SUPPRESS *HETERODERA* POPULATIONS

M. I. Hamid^{1*}, S. Aslam¹, A. Safdar¹, M. Z. Majeed², M. A. Javed³, and A. Mehmood¹

¹Department of Plant Pathology, College of Agriculture, University of Sargodha, Sargodha, 40100, Pakistan; ²Department of Entomology, College of Agriculture, University of Sargodha, Sargodha, 40100, Pakistan; ³Department of Agricultural Extension, College of Agriculture, University of Sargodha, Sargodha, 40100, Pakistan; *Corresponding author: imran.hamid@uos.edu.pk

ABSTRACT

Hamid, M. I., S. Aslam, A. Safdar, M. Z. Majeed, M. A. Javed, and A. Mehmood. 2023. Distribution of cereal cyst nematodes in rice fields of Punjab, Pakistan and evaluation of nematicidal potential of rhizobacteria to suppress *Heterodera* populations. *Nematropica* 53:110-119.

Cereal cyst nematodes (CCN) are among the most devastating endoparasitic sedentary nematodes especially, *Heterodera avenae* and *Heterodera oryzae*, which attack a wide range of small grain crops. A survey was conducted in 2020 to determine the CCN infestation in the four main rice-growing districts of Punjab, Pakistan. Cereal cyst nematodes were found in 38.5% of the samples collected. Seven bacterial species isolated from the rhizosphere of different crop plants were evaluated against CCN. Among the seven bacterial species, *Pseudomonas fluorescens*, *Bacillus subtilis*, *Pseudomonas putida*, and *Pseudomonas geniculata* caused more than 70% CCN second-stage juvenile (J2) mortality when applied as bacterial cultures, culture filtrates, and volatiles. Egg hatching of CCN was also reduced with the application of bacterial cultures, culture filtrates, and volatiles. *P. fluorescens*, *P. geniculata* and *B. licheniformis* significantly reduced egg hatching to 20% compared to the untreated control in which hatching was 65% after 72 hr. The results revealed that rhizospheric bacteria have the potential to kill CCN J2 and reduce egg hatch. These bacterial species have the potential to be alternatives to chemical nematicides for the eco-friendly management of CCN.

Key words: Antagonism, cereal cyst nematode, egg hatching, nematode mortality, rhizobacteria

RESUMEN

Hamid, M. I., S. Aslam, A. Safdar, M. Z. Majeed, M. A. Javed, and A. Mehmood. 2023. Distribución del nematodo del quiste de los cereales en campos de arroz en Punjab, Pakistan y evaluación del potencial nematicida de rhizobacteria para suprimir poblaciones de *heterodera*. *Nematropica* 53:110-119.

Los nematodos del quiste de los cereales (CCN) se encuentran entre los nematodos endoparásitos sedentarios más devastadores, especialmente *Heterodera avenae* y *Heterodera oryzae*, que atacan una amplia gama de cultivos de cereales menores. En el 2020 se realizó un estudio para determinar la infestación del CCN en cuatro distritos principales productores de arroz de Punjab, Pakistán. El nematodo del quiste de los cereales se encontró en el 38,5% de las muestras recolectadas. Se evaluaron siete especies de bacterias

aisladas de la rizosfera de diferentes cultivos, contra el CCN que infecta el arroz. Entre las siete especies bacterianas, *Pseudomonas fluorescens*, *Bacillus subtilis*, *Pseudomonas putida* y *Pseudomonas geniculata* causaron más del 70% de mortalidad en juveniles de segunda etapa (J2) de CCN cuando se aplicaron como cultivos bacterianos, cultivos filtrados y volátiles. La eclosión de huevos de CCN también se redujo con la aplicación de cultivos bacterianos, cultivos filtrados y volátiles. *P. fluorescens*, *P. geniculata* y *B. licheniformis* redujeron significativamente la eclosión de los huevos a 20 % en comparación con el control sin tratamiento en el que la eclosión fue del 65 % después de 72 h. Los resultados revelaron que las bacterias rizosféricas tienen el potencial de matar a CCN J2 y restringir la eclosión de los huevos. Estas especies bacterianas tienen el potencial de ser alternativas a los nematocidas químicos para el manejo ecológico de CCN.

Palabras clave: Antagonismo, nematodo del quiste de los cereales, eclosión de huevos, mortalidad de nematodos, rizobacteria

INTRODUCTION

Cereal grains are one of the main staple foods that feed humans across the world. Additionally, cereal crops are used by the beverage industry and as animal feed due to their high nutritional value (Newman and Newman, 2008; Gustafson *et al.*, 2009). Rice (*Oryza sativa* L.) is an important grain crop and a primary food source for half of the world's population (Subudhi *et al.*, 2006). In Pakistan rice is cultivated on 3 million ha with an annual production of 7.4 million tons (Pakistan Bureau of Statistics, 2020). A decline in production of small grains was observed starting in 2005, and is projected to continue while demand will increase by 1% annually (Alexandratos and Bruinsma, 2012; OECD-FAO, 2018). The factors responsible for the decline in the production of cereal crops must be addressed in a timely manner.

Cereal cyst nematodes (CCN; *Heterodera* spp.) are among the most important pests that limit the production of small grains. CCN invade young plant roots, resulting in stunted growth and chlorotic patches in the fields. CCN belong to the genus *Heterodera*, which is comprised of as many as 70 species, including a complex of 12 species known as the *Heterodera avenae* group (Smiley and Yan, 2010). CCN have been reported to reduce the yield of cereal grains by as much as 20% in Pakistan, 50% in Turkey, 50% in Australia, and 90% in Saudi Arabia (Abidou *et al.*, 2005; Elekçioğlu *et al.*, 2009; Iley and McKay, 2009; Dababat *et al.*, 2015a). More than 50% incidence of CCN have been recorded in surveyed fields in the USA, Iran, Turkey, and Europe (Rivoal and Cook, 1993; Smiley *et al.*, 1994; Tanha *et al.*,

2009). Population densities of plant-parasitic nematodes have a negative relationship with yield, and this relationship is influenced mainly by nematode population densities in a particular area, variety, climate conditions, cultural practices, and soil health (Ali *et al.*, 2019). With the potential for substantial yield loss of grain crops by CCN, proper management practices need to be adopted.

Management of CCN has mostly relied on chemical pesticides, but due to the toxic effects on human health, environment, and water sources, the concerns about the use of nematicides and fumigants are increasing (Dababat *et al.*, 2015b; Damalas and Koutroubas, 2016). Moreover, in highly intensive production systems, chemical-based management strategies impose extra financial burden on growers (Sorribas *et al.*, 2005). The development of new and alternative approaches for nematode management is therefore required. Biological control with bacteria has the potential to efficiently manage plant-parasitic nematodes in an environmentally friendly manner and the potential to reduce nematode densities and resultant damage to plants (Ashoub and Amara, 2010; Basyony and Abo-Zaid, 2018; Soliman *et al.*, 2019). Several beneficial microorganisms have shown potential and have been successfully used as biocontrol agents to manage plant-parasitic nematodes on different crops (Affokpon *et al.*, 2011). Several bacteria such as *Bacillus thuringiensis*, *Pseudomonas fluorescens*, and *Pasteuria penetrans* have exhibited great potential as biological control agents of CCN (Davies, 1998; Siddiqui and Mahmood, 1999). Several beneficial bacteria are known to suppress plant-parasitic nematodes by producing secondary metabolites

such as enzymes and toxins, which could inhibit nematode reproduction, reduce egg hatching, and cause juvenile mortality (Siddiqui and Mahmood, 1999; Zhai *et al.*, 2018). Thus, it is important to isolate and identify native bacterial strains that are well adapted to local environmental conditions.

The first objective of this study was to assess the population densities of CCN in four of the major rice-growing districts of Punjab, Pakistan. The second objective was to evaluate the nematicidal potential of native rhizospheric bacteria against CCN under laboratory conditions.

MATERIALS AND METHODS

Survey of CCN from rice fields

A survey of CCN was conducted during 2020 in the districts of Hafizabad, Shekhupura, Narowal, Gujranwala of Punjab Province, Pakistan (Fig. 1). Soil and root samples were collected by selecting 10 rice fields from each district. From each field, five soil/root samples were collected at a 5-30 cm depth around the root zone following a cross sampling method. A total of 200 soil/root samples were collected from 40 rice fields. The collected soil and root samples were placed in bags and transferred to the laboratory for further processing.

Soil samples were homogenized and 100 g of soil from each sample was processed to extract

cysts and second-stage juveniles (J2) by Cobb's decanting and flotation method (Cobb, 1918). The soil samples were completely mixed with water and passed through sieves with cyst retained on the 148- μ m aperture sieve and J2 retained on the 43- μ m aperture sieve. The cysts and J2 were further extracted by the sucrose flotation method after centrifugation (Hamid *et al.*, 2017). The eggs of CCN were released from cysts by using 5 ml glass tissue grinder and subsequently suspended in a 37% sucrose solution and collected on a 25- μ m aperture sieve after centrifugation at 2500 \times g for 5 min.

The extracted cysts from each sample were observed under a stereomicroscope (Micros, Glan, Austria) and counted to determine the CCN population density. The number of eggs and J2 in each sample were also counted by placing 1 ml of suspension into 12-well tissue culturing plates under an inverted microscope (Olympus CK40, Evident, Tokyo, Japan). The *Heterodera* spp. were determined by observing the morphological characteristics of cyst, vulval cone, and J2 by light microscopy (Micros, Austria) (Mwesige *et al.*, 2020). Significant differences between CCN densities across the four locations were evaluated using ANOVA and LSD test by comparing the treatment means by using Statistcs 8.1 package. The treatments were considered significant when $P < 0.05$.

Evaluation of bacterial strains for CCN suppression

Rhizosphere bacterial strains used in this study were isolated from different host plants (Table 1) during 2017-2020 and maintained in 15% glycerol solution at -80°C. The bacterial strains were cultured on nutrient agar plates for 48 hr at 25°C prior to use in the experiments. Two loops full of bacteria from pure cultures were collected and inoculated in 250-ml flask containing 100 ml of sterilized nutrient broth. The flasks were placed on a shaking incubator at 150 rpm for 72 hr at 28°C in the dark. The bacterial cell suspension was transferred to 50-ml falcon tubes, and centrifuged at 9000 rpm for 10 min. The culture filtrates were transferred to fresh 50 ml falcon tubes and cell pellets were washed with sterilized distilled water followed by centrifugation. The culture filtrates were passed through 40 μ m bacterial filters to obtain cell free filtrates. The bacterial cell cultures



Figure 1. Geographical locations of rice-growing regions of Punjab province, Pakistan

Table 1. Rhizosphere bacterial strains used in the study.

Serial No.	Isolate No.	Bacterial strain	Accession No.
1	CA09	<i>Bacillus licheniformis</i>	MT380164
2	CA26	<i>Pseudomonas fluorescens</i>	MT197384
3	CA27	<i>Bacillus subtilis</i>	MT197385
4	CA31	<i>Oceanobacillus kimchi</i>	MT380165
5	CA34	<i>Rhizobium pusense</i>	MT197388
6	CA41	<i>Pseudomonas putida</i>	MT197386
7	CA62	<i>Pseudomonas geniculata</i>	MT197387

were suspended in sterilized distilled water to achieve a concentration of 1×10^8 cells/ml. The bacterial cell suspension and filtrates were used immediately in nematode parasitism assays.

Healthy CCN J2 were extracted by following Whitehead and Hemming tray method (Whitehead and Hemming, 1965). The extracted J2 were surface sterilized with 0.5% NaOCl and then washed three times with sterilized distilled water. The bacterial cell cultures (1×10^8 cells/ml) and culture filtrates (20%) were used in nematode parasitism assays. In 12-well tissue culture plates, 100 surface-sterilized J2 were added to wells containing the bacterial cultures. The volatile activity of bacterial strains was also accessed by adding 1-ml of culture filtrates in a sterilized glass cup that was placed in the center of a petri dish containing healthy CCN J2 in sterilized distilled water. The untreated control of sterilized distilled water was included in each assay. The bacterial cultures and volatile compounds were replicated four times, and the experiments were conducted twice. CCN J2 mortality by bacterial cell cultures, culture filtrates and volatiles was monitored after 48 hr of incubation at 28°C. The eggs of CCN were released from healthy cysts by using a 5-ml glass tissue grinder and collected in sterilized distilled water. The eggs were surface sterilized using 0.5% NaOCl with three subsequent washings with sterilized water. The bacterial cell cultures (1×10^8 cells/ml) and culture filtrates (20%) were used for *in vitro* egg hatching assays by adding 100 CCN eggs in each well of 12-well tissue culture plates. The volatile activity of bacterial strains was also accessed by adding 1 ml of culture filtrates in a sterilized glass cup that was placed in the center of petri dish containing healthy CCN eggs. The sterilized distilled water containing 0.05% ZnCl₂ was used as an untreated control in the assays. Treatments were replicated four times and experiments conducted twice. The data for number of eggs hatched after 72 hr were recorded. The data

for percentage J2 mortality and egg hatch were collected and analyzed using ANOVA and LSD test to compare mean values at $P < 0.05$ using Statistix 8.1 software (Tallahassee, FL).

RESULTS

Incidence and population density of CCN across rice growing regions

The CCN species *Heterodera oryzae* and *Heterodera avenae* were detected in all of the rice fields sampled (Fig. 2). Both *Heterodera* spp. were found in mixed populations due to the rice-wheat crop rotation in most of the fields sampled. The average CCN infestation across fields was 38.5%. The highest incidence of CCN was recorded in Hafizabad followed by Narowal with CCN found in 40% and 39% fields, respectively. The lowest incidence was recorded from Gujranwala and Sheikhpura districts with CCN found in 36% of

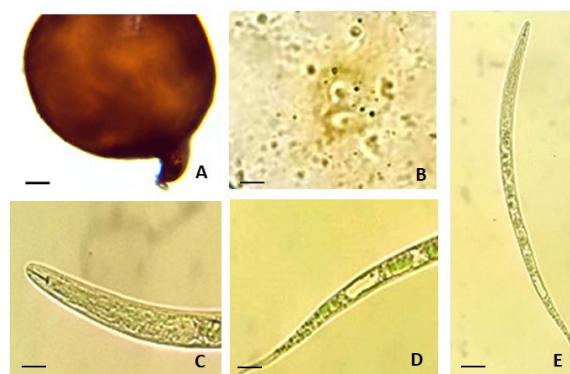


Figure 2. Morphological observations of cereal cyst nematode (CCN) extracted from rice fields. A) cyst of CCN, B) vulva cone of CCN, C) head region of juvenile of CCN, D) tail region of CCN, E) full body of CCN.

surveyed fields. The mean population densities of CCN across rice fields were 36 and 26 cysts/100 g soil in Hafizabad and Narowal, respectively. Moreover, 25 cysts/100 g soil were found in Gujranwala and Sheikhpura districts (Fig. 3A). The statistical analysis revealed that only Hafizabad was statistically different in cyst population densities from Sheikhpura, Narowal and Gujranwala ($P < 0.05$). The mean number of eggs and J2 per 100 g soil was 1,162 and 940 from Hafizabad and Sheikhpura districts, respectively. Fewer CCN eggs and J2 were detected in Narowal and Gujranwala districts with 865 and 810 eggs and J2 per 100 g soil, respectively (Fig. 3B). CCN egg and J2 population densities were significantly higher in Hafizabad and then Sheikhpura compared to Narowal and Gujranwala ($P < 0.05$).

Nematicidal activity of bacteria against CCN J2

The bacterial strains *P. fluorescens* CA26, *B. subtilis* CA27, *P. putida* CA41, and *P. geniculata* CA62 resulted in the highest CCN mortality of more than 80% in cell culture assays while other bacterial strains resulted in 60% or more CCN

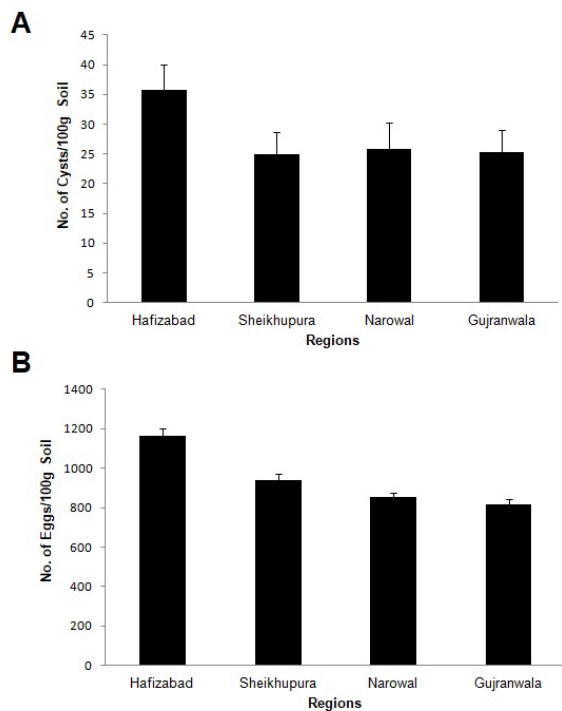


Figure 3. Distribution and incidence of cereal cyst nematode in rice-growing regions of Punjab province, Pakistan. A) Number of cysts/100 g dry soil, B) number of eggs and juveniles/100 g dry soil.

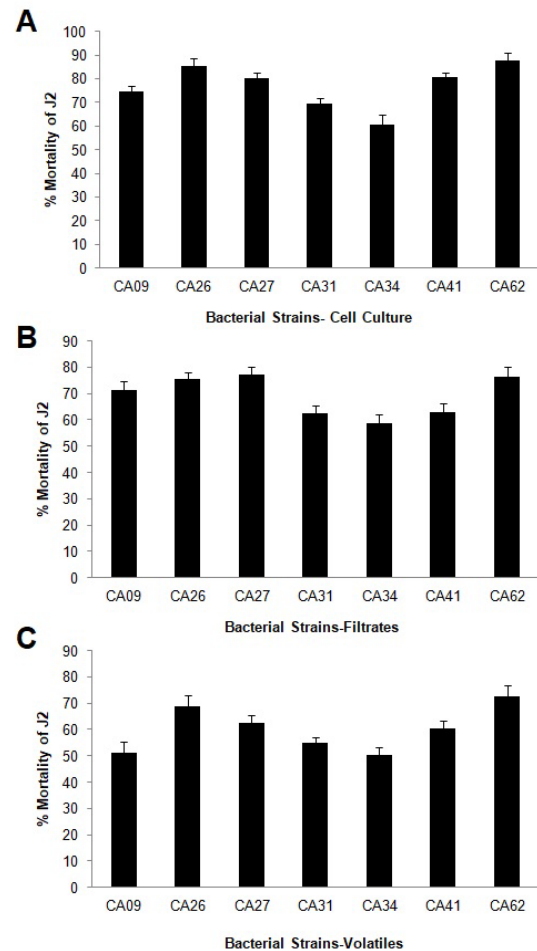


Figure 4. Nematicidal activity of bacterial strains against cereal cyst nematode. A) Percentage mortality of juveniles by bacterial cell culture, B) percent age mortality of juveniles by bacterial culture filtrates, C) percentage mortality of juveniles by bacterial volatiles.

mortality (Fig. 4A). *Pseudomonas fluorescens* CA62 caused higher J2 mortality compared to *P. fluorescens* CA26 and *P. putida* CA41 and the rest of the strains ($P < 0.05$). In the culture filtrates assay, *B. licheniformis* CA09, *P. fluorescens* CA2, *B. subtilis* CA27, and *P. geniculata* CA62 resulted in more than 70% CCN mortality while other bacterial strains resulted in more than 58% CCN mortality (Fig. 4B). *P. fluorescens* CA26, *B. subtilis* CA27, and *P. geniculata* CA62 caused significantly higher mortality of J2 than other bacterial strains and the untreated control ($P < 0.05$).

The volatile effects of bacterial culture filtrates revealed that *P. fluorescens* CA26, *B.*

subtilis CA27, *P. putida* CA41, and *P. geniculata* CA62 caused more than 60% J2 mortality while other bacterial strains caused more than 50% mortality (Fig. 4C). The volatiles of *P. fluorescens* CA62 caused significantly higher J2 mortality than other bacterial strain and the untreated control ($P < 0.05$). Overall, *P. geniculata* CA62 was the most efficient bacterial strain with high nematicidal potential followed by *P. fluorescens* CA26 and *P. putida* CA41.

Efficiency of bacteria on egg hatching of CCN

Inoculation of CCN eggs into bacterial cell cultures significantly reduced CCN egg hatching ability and destroyed most of the eggs. The bacterial strains *P. fluorescens* CA26 and *P. geniculata* CA62 significantly reduced egg hatching by 20% followed by *B. subtilis* CA27 compared to the control in which 65% of eggs hatched in 72 hr (Fig. 5A). The assays with culture filtrates also reduced CCN egg hatching significantly compared to the control. *P. geniculata* CA62 and *B. licheniformis* CA09 caused significantly higher reduction in egg hatching, 22 and 24.5%, respectively, followed by *B. subtilis* CA27 compared to the control (Fig. 5B). The volatiles of bacterial filtrates also reduced egg hatching of CCN significantly compared to the untreated control. *P. geniculata* CA62 and *P. fluorescens* CA26 had the highest reduction in egg hatching followed by *P. putida* CA41 compared to the control. All other bacterial strains also resulted in significant reductions in egg hatching compared to the untreated control (Fig. 5C). *P. geniculata* CA62 and *P. fluorescens* CA26 were the most efficient bacterial strains in reducing egg hatching in all assays compared to the other bacterial strains and the untreated control ($P < 0.05$).

DISCUSSION

The present study was conducted to investigate the distribution of CCN in the four main rice-growing districts of Punjab province, Pakistan. In these regions, rice-wheat monoculture is adopted on most of the land. The results revealed the presence of CCN in most of the fields sampled with 25-36 cysts and 810-1,210 eggs and J2 per 100 g soil. The two main species, *H. avenae* and *H. oryzae*, were detected in the rice fields. The incidence and species diversity of CCN were

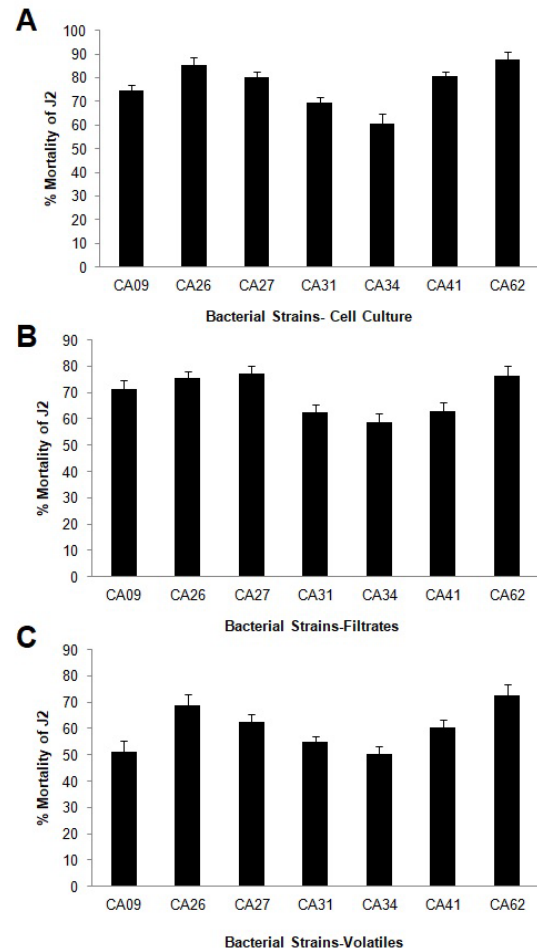


Figure 4. Nematicidal activity of bacterial strains against cereal cyst nematode. A) Percentage mortality of juveniles by bacterial cell culture, B) percentage mortality of juveniles by bacterial culture filtrates, C) percentage mortality of juveniles by bacterial volatiles.

reported from wheat-rice growing regions of Punjab, Pakistan (Hamid *et al.*, 2021) and CCN population densities were lower due to the non-host cropping pattern. Ahmadi and Maafi (2014) studied the incidence of CCN (*H. avenae* and *H. filipjevi*) in different regions of southwestern Iran and showed varying degrees of nematode infestation. It was also observed that CCN have more damaging effects in rain-fed cereal crops compared to the cereal crops grown in irrigated lands (Smiley, 2009). Variation in CCN infestation may be due to different factors like the application of nematicides, soil solarization, cultural practices, or the presence of fungal and bacterial antagonists (Chen, 2007). The results revealed that most of the

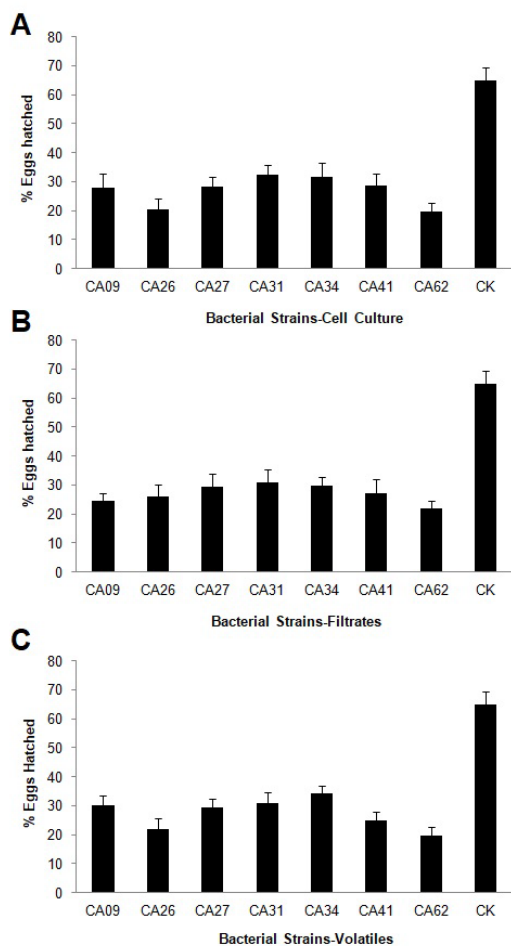


Figure 5. Reduction in egg hatching of cereal cyst nematode. A) Percentage egg hatching by bacterial cell culture, B) percentage egg hatching by bacterial culture filtrates, C) percentage egg hatching by bacterial volatiles.

rice fields were infested with CCN and proper management practices should be considered.

In this study, already characterized rhizosphere bacterial strains were evaluated against CCN using cell culture, culture filtrates and volatile assays. Egg-hatching capacity was decreased by treating CCN eggs with bacterial cell culture, filtrates, and volatiles. The bacterial strains resulted in 60-90% nematode mortality and 50-70% reduction in egg hatching. Several bacterial and fungal species recovered from the rhizosphere have been shown to play a vital role in the suppression of plant-parasitic nematodes (Gao *et al.*, 2016). A study demonstrated the nematicidal potential of bacterial and fungal isolates from plant rhizosphere against *Meloidogyne* sp. of soybean (Toju and Tanaka, 2019). The thorough investigation of the

nematicidal potential of *B. firmus* against plant-parasitic nematodes revealed the effect of a serine protease enzyme with high nematode suppression (Geng *et al.* 2016). The cytotoxic effect of *P. fluorescens* strain UTPF5 killed *Meloidogyne javanica* J2 and completely eliminated egg hatching (Bagheri *et al.*, 2014). The wettable formulation of *Pseudomonas* sp. O6 was used to control fungal diseases by foliar application and also provided preventive and curative protection against *Meloidogyne* spp. in tomato (Nam *et al.*, 2018). The biological potential of bacteria against CCN and other plant-parasitic nematodes is well documented and may be a good alternative to chemical nematicides.

The production of multiple enzymes in the form of antibiotics by rhizobacteria is the key weapon for biological activity against plant-parasitic nematodes. The production of antibiotics by rhizosphere bacterial strains, such as pyoluteorin, pyrrolnitrin, 2,4-diacetylphloroglucinol, and phenazine were the major determinants of the biocontrol activity of *Pseudomonads* (Bernal *et al.*, 2017). Several volatile compounds such as, dimethyl-disulfide, 2-nonanone, 2-undecanone, 2-octanone, 1-undecene, 1-(ethenyloxy)-octadecane, and (Z)-hexen-1-ol acetate were identified from a *P. putida* strain having strong nematicidal activity against *M. incognita* J2 and eggs (Zhai *et al.*, 2018). The volatile compounds, dimethyl-disulfide, 2-nonanone, and 2-undecanone, caused more than 80% reduction of both *M. incognita* J2 and eggs at a very low concentration of 0.5 mmol after 7 days of exposure (Huang *et al.*, 2010). In another study, dimethyl-disulfide exhibited the strongest nematicidal activity against *Bursaphelenchus xylophilus* (Yu *et al.*, 2015). The compounds 2-nonanone and 2-undecanone were reported to induce paralysis in *M. incognita* and *M. javanica* by direct-contact (Ntali *et al.*, 2011). The repellent effect of bacterial filtrates at high concentrations against *M. incognita* has been demonstrated (Zhai *et al.*, 2018). Volatiles have also been demonstrated to attract nematodes. For example, *B. nematocida* B16 attracted nematodes by releasing six different volatile compounds, of which benzaldehyde, benzyl benzoate, acetophenone and 2-heptanone were potent attractants (Niu *et al.*, 2010). In future studies, nematicidal compounds produced by these rhizospheric bacterial strains considered in this study should be characterized.

In conclusion, this study showed the distribution of CCN in the main four rice-growing districts of Punjab, Pakistan. The efficacy of native rhizosphere bacteria was evaluated under *in vitro* conditions and *P. geniculata*, *P. fluorescens* and *B. subtilis* were shown to have high potential to cause CCN J2 mortality and reduce egg hatching compared to other bacterial strains and the untreated control. These bacterial strains will be further evaluated in greenhouse and field experiments. Moreover, nematicidal compounds produced by these bacteria will also be characterized.

ACKNOWLEDGMENTS

The funding for the research was provided by University of Sargodha under ORIC-UOS projects (UOS/ORIC/2016/16).

LITERATURE CITED

- Abidou, H., A. El-Ahmed, J. M. Nicol, N. Bolat, R. Rivoal, and A. Yahyaoui. 2005. Occurrence and distribution of species of the *Heterodera avenae* group in Syria and Turkey. *Nematologia Mediterranea* 33:195–201.
- Affokpon, A., D. L. Coyne, C. C. Htay, R. D. Agbèdè, L. Lawouin, and J. Coosemans. 2011. Biocontrol potential of native *Trichoderma* isolates against root-knot nematodes in West African vegetable production systems. *Soil Biology and Biochemistry* 43:600–608.
- Ahmadi, A. R., and Z. Tanha Maafi. 2014. Incidence of cereal cyst nematodes (*Heterodera avenae* type B and *H. filipjevi*) in southwestern Iran. *Journal of Crop Protection* 3:75–88.
- Alexandratos, N., and J. Bruinsma. 2012. In: *World Agriculture towards 2030/2050: The 2012 Revision*. Food and Agriculture Organization of the United Nations, New York, USA.
- Ali, M. A., M. Shahzadi, A. Zahoor, A. A. Dababat, H. Toktay, A. Bakhsh, M. A. Nawaz, and H. Li. 2019. Resistance to cereal cyst nematodes in wheat and barley: An emphasis on classical and modern approaches. *International Journal of Molecular Science* 20:432. <https://doi.org/10.3390/ijms20020432>
- Ashoub, A. H., and M. T. Amara. 2010. Biocontrol activity of some bacterial genera against root-knot nematode, *Meloidogyne incognita*. *Journal of American Science* 6:321–328.
- Bagheri, N., M. Ahmadzadeh, and R. Heydari. 2014. Effects of *Pseudomonas fluorescens* strain UTPF5 on the mobility, mortality and hatching of root-knot nematode *Meloidogyne javanica*. *Archive of Phytopathology and Plant Protection* 47:744–752.
- Basyony, A. G., and G. A. Abo-Zaid. 2018. Biocontrol of the root-knot nematode, *Meloidogyne incognita*, using an eco-friendly formulation from *Bacillus subtilis*, lab. and greenhouse studies. *Egyptian Journal of Biological Pest Control* 28:1–13.
- Bernal, P., L. P. Allsopp, A. Filloux, and M. A. Llamas. 2017. The *Pseudomonas putida* T6SS is a plant warden against phytopathogens. *ISME Journal* 11:972–987.
- Chen, S. 2007. Suppression of *Heterodera glycines* in soils from fields with long-term soybean monoculture. *Biocontrol Science and Technology* 17:125–134.
- Cobb, N. A. 1918. Estimating the nema population of soil, with special reference to the sugar-beet and root-gall nemas, *Heterodera schachtii* Schmidt and *Heterodera radiculicola* (Greef) Müller: and with a description of *Tylencholaimus aequalis* n. sp. USDA, Department of Plant Industry, and Office of Agricultural Technology. Washington, DC: US Government Printing Office.
- Dababat, A. A., M. Imren, G. Erginbas-Orakci, S. Ashrafi, E. Yavuzaslanoglu, H. Toktay, and T. Mekete. 2015b. The importance and management strategies of cereal cyst nematodes, *Heterodera* spp., in Turkey. *Euphytica* 202:173–188.
- Dababat, A. A., H. Muminjanov, and R. W. Smiley. 2015b. *Nematodes of Small Grain Cereals: Current Status and Research*. Ankara, Turkey: FAO.
- Damalas, C. A., and S. D. Koutroubas. 2016. Farmers' exposure to pesticides: Toxicity types and ways of prevention. *Toxics* 4:1–10.
- Davies, K. G. 1998. Natural parasites and biological control research. Pp. 369-397 in S.B. Sharma, (ed.) *Cyst-forming Nematodes*. London: Chapman Hall, London.
- Elekçioğlu, İ. H., J. Nicol, N. Bolat, E. Sahin, A. Yorgancılar, H. Braun, O. Yorgancılar, A. Yıldırım, A. Kılınç, H. Toktay, and M. Çalışkan. 2009. Long-term studies on the

- cereal cyst nematode *Heterodera filipjevi* in Turkey: International collaboration with regional implications. Pp. 11-16 in I. T. Riley IT, J. M. Nicol, and A. A. Dababat (eds.) Cereal Cyst Nematodes: Status, Research and Outlook. Ankara, Turkey: CIMMYT.
- Gao, H., G., Qi, R. Yin, H. Zhang, C. Li, and X. Zhao. 2016. *Bacillus cereus* strain S2 shows high nematicidal activity against *Meloidogyne incognita* by producing sphingosine. Scientific Reports 6:1–11.
- Geng, C., X. Nie, Z. Tang, Y. Zhang, J. Lin, M. Sun, and D. Peng. 2016. A novel serine protease, Sep1, from *Bacillus firmus* DS-1 has nematicidal activity and degrades multiple intestinal-associated nematode proteins. Scientific Reports 6:1–12.
- Gustafson, P., O. Raskina, M. Xuefeng, and N. Eviatar. 2009. Wheat evolution, domestication, and improvement. Wheat Science and Trade, Wiley-Blackwell.
- Hamid, M. I., M. U. Ghazanfar, M. Q. N. Khan, Z. ul Hasan, and S. Aslam. 2021. Incidence of cereal cyst nematode prevailing in wheat-rice growing regions of Punjab province, Pakistan. Agricultural Science Digest 1–6.
- Hamid, M. I., M. Hussain, Y. P. Wu, X. Zhang, M. Xiang, and X. Z. Liu. 2017. Successive soybean-monoculture cropping assembles rhizosphere microbial communities for the soil suppression of soybean cyst nematode. FEMS Microbiology Ecology 93:1–10.
- Huang, Y., C. Xu, L. Ma, K. Zhang, C. Duan, and M. Mo. 2010. Characterization of volatiles produced from *Bacillus megaterium* YFM3. 25 and their nematicidal activity against *Meloidogyne incognita*. European Journal of Plant Pathology 126:417–422.
- Iley, I. T., and A. McKay. 2009. Cereal cyst nematode in Australia: Biography of a biological invader. Pp. 23-28 in I. T. Riley, J. M. Nicol, and A. A. Dababat, eds. Cereal Cyst Nematodes: Status, Research and Outlook. Ankara, Turkey: CIMMYT.
- Mwesige, R., E. H. Kim, E. H. Park, and H. R. Ko. 2020. Morphological and molecular characterizations of *Heterodera oryzae* in Korea. Journal of Nematology 52: 1–12.
- Nam, H. S., A. J. Anderson, and Y. C. Kim. 2018. Biocontrol efficacy of formulated *Pseudomonas chlororaphis* O6 against plant diseases and root-knot nematodes. Plant Pathology Journal 34:241.
- Newman, R. K., and C. W. Newman. 2008. Barley history: relationship of humans and barley through the ages. Pp. 1-18 in R. K. Newman, and W. Newman, eds. Barley for Food and Health. Science, Technology and Products. New Jersey: John Wiley and Sons, Inc.
- Niu, Q., X. Huang, L. Zhang, J. Xu, D. Yang, K. Wei, X. Niu, Z. An, J. W. Bennett, C. Zou, J. Yang, and K. Q. Zhang. 2010. A Trojan horse mechanism of bacterial pathogenesis against nematodes. PNAS 107:16631–16636.
- Ntalli, N. G., F. Manconi, M. Leonti, A. Maxia, and P. Caboni. 2011. Aliphatic ketones from *Ruta chalepensis* (Rutaceae) induce paralysis on root knot nematodes. Journal of Agriculture and Food Chemistry 59:7098–7103.
- OECD-FAO. 2018. Agricultural Outlook Organisation for Economic Cooperation and Development (OECD) and Food and Agricultural Organization of the United Nations (FAO). OECD Agricultural Statistics (<https://www.oecd.org/publications/oecd-fao-agricultural-outlook-19991142.htm>).
- Pakistan Bureau of Statistics. 2020. Government of Pakistan, Islamabad, Pakistan.
- Rivoal, R., and R. Cook. 1993. Nematode pests of cereals. Pp. 259-303 in K. Evans, D. L. Trudgill, and J. M. Webster, eds. Plant parasitic nematodes in temperate agriculture. Wallingford, UK: CAB International.
- Siddiqui, Z. A., and I. Mahmood. 1999. Role of bacteria in the management of plant parasitic nematodes: A review. Bioresource Technology 69:167–179.
- Smiley, R. W. 2009. Occurrence, distribution and control of *Heterodera avenae* and *H. filipjevi* in the western USA. Pp. 35-40 in I. T. Riley, J. M. Nicol, and A. A. Dababat, eds. Cereal Cyst Nematodes: Status, Research and Outlook. Ankara, Turkey: CIMMYT.
- Smiley, R. W., R. E. Ingham, W. Uddin, and G. H. Cook. 1994. Crop sequences for managing cereal cyst nematode and fungal populations of winter wheat. Plant Disease 78:1142–1149. Smiley, R. W., and J. M. Nicol. 2009. Nematodes which challenge global wheat production. Wheat Science and Trade 171–187.
- Smiley, R. W., and G. Yan. 2010. Cereal cyst nematodes: Biology and management in Pacific Northwest wheat, barley, and oat

- crops. OSU Extension Catalog.
- Soliman, G. M., H. H. Ameen, S. M. Abdel-Aziz, and G. M. El-Sayed. 2019. In vitro evaluation of some isolated bacteria against the plant parasite nematode *Meloidogyne incognita*. *Bulletin of National Research Centre* 43:1–7.
- Sorribas, F. J., C. Ornat, S. Verdejo-Lucas, M. Galeano, and J. Valero. 2005. Effectiveness and profitability of the Mi-resistant tomatoes to control root-knot nematodes. *European Journal Plant Pathology* 111:29–38.
- Subudhi, P. K., T. Sasaki, and G. S. Khush. 2006. Rice. Pp. 1-78 in C. Kole, ed. *Genome mapping and molecular breeding in plants (1), Cereals and millets*. Berlin, Heidelberg: Springer-Verlag.
- Tanha, M. Z., J. M. Nicol, H. Kazemi, N. Ebrahimi, M. Gitty, M. Ghalandar, M. Mohammadi-Pour, and Z. Khoshkhabar. 2009. Cereal cyst nematodes, root rot pathogens and root lesion nematodes affecting cereal production in Iran. Pp. 51-55 in I. T., Riley, J. M. Nicol, and A. A. Dababat, eds. *Cereal Cyst Nematodes: Status, Research and Outlook*. Ankara, Turkey: CIMMYT.
- Toju, H., and Y. Tanaka. 2019. Consortia of anti-nematode fungi and bacteria in the rhizosphere of soybean plants attacked by root-knot nematodes. *Royal Society of Open Science* 6:181693.
- Whitehead, A. G., and J. R. Hemming. 1965. A comparison of some quantitative methods of extracting small vermiform nematodes from soil. *Annals of applied Biology* 55: 25-38.
- Yu, J., G. Du, R. Li, L. Li, Z. Li, C. Zhou, and D. Guo. 2015. Nematicidal activities of bacterial volatiles and components from two marine bacteria, *Pseudoalteromonas marina* strain H-42 and *Vibrio atlanticus* strain S-16, against the pine wood nematode, *Bursaphelenchus xylophilus*. *Nematology* 17:1011–1025.
- Zhai, Y., Z. Shao, M. Cai, L. Zheng, G. Li, D. Huang, and J. Zhang. 2018. Multiple modes of nematode control by volatiles of *Pseudomonas putida* 1A00316 from Antarctic soil against *Meloidogyne incognita*. *Frontiers in Microbiology* 9:253.

Received:

16/IV/2023

Recibido:

Accepted for publication:

26/IX/2023

Aceptado para publicación: