Emerging strategy for eco-friendly management of black pepper root-knot nematodes

Van Bon Nguyen¹,†, San-Lang Wang²,*, Thi Hanh Nguyen²,³,†, Anh Dzung Nguyen¹,*

¹Institute of Biotechnology and Environment, Tay Nguyen University, 567 Le Duan Str., Buon Ma Thuot City 630000, Viet Nam
²Department of Chemistry, Tamkang University, 151 Yingzhuang Rd., Tamsui Dist., New Taipei City 25137, Taiwan
³Doctoral Program in Applied Sciences, Tamkang University, New Taipei City 25137, Taiwan

*Emails: sabulo@mail.tku.edu.tw, nadzung@ttu.edu.vn
†Equal contribution to this paper

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Abstract. Black pepper (Piper nigrum L.) is one of the most important industrial crops with high economic value. This crop is cultivated in Viet Nam, Indonesia, India, and Brazil. Of these, Viet Nam is the largest producer and exporter of peppercorns in the world. However, the cultivation of this plant has faced various pathogen diseases, including the root-knot nematodes (RKNs) causing the effect on quality and productivity of peppercorns. Many methods have been investigated for controlling and preventing this disease. The number of studies on screening and utilization of beneficial microbes for the effective treatment of RKNs has increased and is also recognized as an emerging research topic. Recently, various RKNs-related reviews have been reported. However, there are only a few overview papers concerning the eco-friendly management of black pepper RKNs via using beneficial microbes and secondary metabolites. This review extensively presents, discusses and emphasizes the significant research results concerning this novel strategy for the effective management of black pepper RKNs, as well as future studies in this direction. This review provides complete scientific information which may be useful for further investigation of sustainable cultivation of black pepper crops.

Keywords: Black pepper, root-knot nematodes, beneficial microbes, nematocidal compounds, microbial fermentation.

Classification numbers: 1.1, 2.3.

1. INTRODUCTION

Black pepper is an essential crop with high economic value for export worldwide. Its product, peppercorn, has been used daily as the most widely traded spice reaching approximately 20 % of all global spices [1. 2]. This plant has been widely cultivated in several countries, including Viet Nam, Indonesia, India, and Brazil. Of these, Viet Nam is the largest producer and exporter of peppercorns, with approximately 40 % of 546,000 tons [3]. The Central Highlands
and southeastern areas produce the highest amount of black peppercorns in Viet Nam, with about 124.5 ha and a production of 193.3 tons [4]. However, this spicy plant crop faces various pathogens, including root-knot nematodes (RKNs) [5 - 13]. One of the major nematodes that cause severe damage to black pepper is *Meloidogyne incognita* species [14].

Up to now, various methods have been investigated and applied for the management of RKNs, such as chemical methods [15, 16] and green approaches, including using living microorganisms, plant extracts, essential oils, and natural inhibitors [6, 16 - 18]. The application of various cultivation methods affects soils or crops [7, 8, 15, 16]. The management of RKNs using beneficial microbial strains and their active secondary metabolites has received significant interest due to their cost-effectiveness and environmental issues.

*Figure 1.* Black pepper plant with yellow leaves shows the symptom of root-knot nematode infection (A). Roots with knots (B) were collected for the isolation of nematodes eggs (C), and J2 nematodes were obtained by incubating eggs after 2 days.
To date, there are only a few review papers concerning the eco-friendly management of RKNs infected on black pepper via using beneficial microbial strains and secondary metabolites. Recently, an overview of the management of black pepper nematodes by using microorganism agents has been published [13]. This previous review summarized and discussed the use of some bacterial and fungal agents for the eco-friendly management of black pepper RKNs [13]. In this work, we updated and discussed the potential use of indigenous microbes for cost-effective management of black pepper RKNs in vitro, under greenhouse and field conditions. The applications of fermentation technology using organic wastes for scaling-up bioproduction of nematocidal compounds for potential use are also discussed in this review. Herein, we also supplied full scientific information on emerging and cost-effective strategies for the treatment of RKNs aiming at sustainable cultivation of black pepper crops.

2. APPLICATION OF BENEFICIAL MICROBES FOR THE MANAGEMENT OF BLACK PEPPER NEMATODES

2.1. Studies on in-vitro screening beneficial microbes

Beneficial microbes have been recognized and evidenced as effective and eco-friendly agents for managing nematodes and other plant pathogens [19]. Recently, considering the sustainable production of black pepper crop, various studies have focused on the isolation and identification of active rhizomicrobes and endophytic microbes and investigation of their potential use for biocontrol of the root-knot nematodes (RKNs) in the Central Highlands of Viet Nam [1, 5, 19 - 26]. The strains were selected and identified based on gene sequencing, and their in vitro nematocidal activities are summarized in Table 1.

Table 1. The potent anti-nematode microbial strains.

<table>
<thead>
<tr>
<th>Microbial Strains</th>
<th>Origin</th>
<th>Anti-nematode activity</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Anti-J2 (%)</td>
<td></td>
</tr>
<tr>
<td>Bacillus velezensis EK7</td>
<td>RS/DL</td>
<td>99</td>
<td>[20]</td>
</tr>
<tr>
<td>Bacillus mojavensis RB.DS33</td>
<td>RS/DN</td>
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<td>ND</td>
</tr>
<tr>
<td>Bacillus subtilis RB.CJ4</td>
<td>RS/DN</td>
<td>87.33</td>
<td>ND</td>
</tr>
<tr>
<td>Bacillus megaterium E7</td>
<td>RS/DL</td>
<td>83.83</td>
<td>ND</td>
</tr>
<tr>
<td>Bacillus cereus E13</td>
<td>RS/DL</td>
<td>90</td>
<td>ND</td>
</tr>
<tr>
<td>Bacillus atrophaeus BMT10</td>
<td>RS/DL</td>
<td>81.67</td>
<td>ND</td>
</tr>
<tr>
<td>Bacillus megaterium BMT11</td>
<td>RS/DL</td>
<td>98.33</td>
<td>ND</td>
</tr>
<tr>
<td>Acinetobacter baumannii BMT15</td>
<td>RS/DL</td>
<td>88.33</td>
<td>ND</td>
</tr>
<tr>
<td>Bacillus pumilus BHV1.2.2</td>
<td>RS/DL</td>
<td>86.67</td>
<td>ND</td>
</tr>
<tr>
<td>Bacillus sp. BHV3.2.2</td>
<td>RS/DL</td>
<td>98.33</td>
<td>ND</td>
</tr>
<tr>
<td>Serratia marcescens TNU2</td>
<td>RS/DL</td>
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<tr>
<td>Bacillus cereus RB.DS.05</td>
<td>RS/DN</td>
<td>97.67</td>
<td>80</td>
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<tr>
<td>Bacillus subtilis RBDL28</td>
<td>RS/DL</td>
<td>96.33</td>
<td>82</td>
</tr>
<tr>
<td>Staphylococcusxylosus RB.DS.29</td>
<td>RS/DN</td>
<td>96.33</td>
<td>81</td>
</tr>
<tr>
<td>Bacillus megaterium RB.DL.31</td>
<td>RS/DL</td>
<td>96.13</td>
<td>70</td>
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</table>
Table 1: (N D): Not detected; (+): showing activity; RS: rhizosphere soil; DL: Dak Lak province; DN: Dak Nong province; GL: Gia Lai province; BPR: Black pepper roots; These microbial strains were isolated from the roots and rhizosphere soils of black pepper roots cultivated in the Central Highlands of Viet Nam.

<table>
<thead>
<tr>
<th>Microbial Strain</th>
<th>Isolation Site</th>
<th>Inhibition Value (%)</th>
<th>Activity</th>
<th>Isolation Site</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus megaterium H11</td>
<td>RS/DL</td>
<td>98.33</td>
<td>ND</td>
<td></td>
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<tr>
<td>Bacillus pumilus C01</td>
<td>RS/GL</td>
<td>97.21</td>
<td>ND</td>
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<tr>
<td>Escherichia coli RB.DL.9</td>
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<td>83.33</td>
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<td>81.67</td>
<td>ND</td>
<td></td>
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<tr>
<td>Bacillus acidicylota B14</td>
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<td>89.03</td>
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<tr>
<td>Acinetobacter baumannii H15</td>
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<td>85.33</td>
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<tr>
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<td>ND</td>
<td></td>
</tr>
<tr>
<td>Bacillus cereus BMT1</td>
<td>RS/DL</td>
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<td>ND</td>
<td></td>
</tr>
<tr>
<td>Bacillus marisfavi BMT2</td>
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<td>81.67</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Bacillus marisfavi BMT3</td>
<td>RS/DL</td>
<td>83.33</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Bacillus megaterium CC05</td>
<td>RS/GL</td>
<td>87.12</td>
<td>ND</td>
<td></td>
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<td>86.22</td>
<td>ND</td>
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<td>BPR/GL</td>
<td>97.78</td>
<td>ND</td>
<td>[26]</td>
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<tr>
<td>EBDC.05</td>
<td>BPR/GL</td>
<td>98.89</td>
<td>ND</td>
<td>[26]</td>
</tr>
<tr>
<td>EBCP.03</td>
<td>BPR/GL</td>
<td>97.78</td>
<td>ND</td>
<td>[26]</td>
</tr>
<tr>
<td>Bacillus flexus DS5</td>
<td>BPR/DN</td>
<td>100</td>
<td>ND</td>
<td>[1]</td>
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<tr>
<td>Bacillus sp. DS8</td>
<td>BPR/DN</td>
<td>100</td>
<td>ND</td>
<td>[1]</td>
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<tr>
<td>Bacillus megaterium DS9</td>
<td>BPR/DN</td>
<td>100</td>
<td>ND</td>
<td>[1]</td>
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<tr>
<td>Bacillus sp. DR10</td>
<td>BPR/DN</td>
<td>100</td>
<td>ND</td>
<td>[1]</td>
</tr>
<tr>
<td>Bacillus sp. DR2</td>
<td>BPR/DN</td>
<td>100</td>
<td>ND</td>
<td>[1]</td>
</tr>
</tbody>
</table>

Note: Table 1: (ND): Not detected; (+): showing activity; RS: rhizosphere soil; DL: Dak Lak province; DN: Dak Nong province; GL: Gia Lai province; BPR: Black pepper roots; These microbial strains were isolated from the roots and rhizosphere soils of black pepper roots cultivated in the Central Highlands of Viet Nam.

A total of 36 strains were screened as effective against J2 nematodes with inhibition values ranging from 81.67 to 100%; among these, 34 strains belonged to the Bacillus genus. Some of these selected strains exerted their effect on nematode egg hatching and showed good inhibition (70 - 82 %) [5]. Notably, several bacterial strains were evidenced as novel anti-nematode bacteria for the first time in these works [1, 5, 22 - 25]. Various fungal and Streptomyces strains were also isolated and accessed for their nematocidal activity. However, they demonstrated weak activity [19]. In the strategies for sustainable production of black pepper crops, the enhancement of plant growth using plant growth-promoting bacteria (PGPB) instead of synthetic fertilizers is also considered. Thus, hundreds of isolates were evaluated for their activities of nitrogen-fixing, phosphate solubilizing, and synthesis of IAA. Thirteen bacterial strains were found to be potent PGPB. PGPB strains were also assigned to the Bacillus genus [19].

Different from the earlier reports [17, 18, 27, 28], some studies [1, 5, 19 - 26] focused on isolating beneficial microbes living in the black pepper roots and rhizosphere soils of this spice in the Central Highlands of Viet Nam. All the strains were isolated from the healthy black pepper trees surrounded by sick pepper trees. With the same pepper seedlings grown under the same conditions, some trees remained healthy and unaffected by nematodes. This might be due to beneficial microbes in the soil surrounding the roots or living in the roots themselves [1, 5]. This could be considered a novel and effective strategy for the isolation and screening of active and suitable strains for the sustainable cultivation of black pepper.
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Several studies have also screened and evaluated the anti-RKNs activity of various microorganism strains. *Bacillus thuringiensis* showed effective management of *Meloidogyne* sp. infesting on pepper, with the ability to kill up to 100% of J2s and 89.67% of eggs after 10 h treatment at a concentration of $10^9$ cells/mL [29]. Leong et al. evaluated the effect of *Purpureocillium lilacinum* fungi against various stages of development of *Meloidogyne incognita* nematode [30]. Strains A, B, and M of *Purpureocillium lilacinum* showed high rates of parasitism on female nematodes (over 90%) and eggs (66.0-78.8%). The egg-hatching rate was reduced by 89%. However, they were not effective on J2s with low mortality, around 5.5 - 6%. Some *Paecilomyces lilacinus* fungi were tested for colonizing female nematodes [31]. Among them, strains PLA and PLB both showed a high effect on females with parasitism rates over 90% and parasitism rates on eggs from 66 to 78%. Moreover, the egg-hatching rate was significantly reduced after 7 days with significant inhibitory percentages of 88 - 89% compared to the control PLM, reaching only 26% [31]. *Verticillium chlamydosporium* fungi were isolated from an infested black pepper in India. This fungus inhibited the hatching of *Meloidogyne incognita* eggs by 41.4% within 5 days *in vitro* tests [32]. The effect of *Pasteuria* sp. - an obligate parasite of plant-parasitic nematodes for managing harmful *Meloidogyne incognita* on black pepper in India was tested. Female nematodes parasitized by *Pasteuria* lost their ability to reproduce. Besides, it was found that endospores were attached to the nematode cuticle at the highest number in a conventional type of attachment (87.62%), followed by inverted (6.55%) and sideway attachments (5.82%) [33]. In a report by Priyank et al., *Pasteuria* spp. was isolated from soils in India, showing its parasite ability on *Meloidogyne incognita* nematode and completely prohibiting fecundity of the nematode [34].

Overall, the above presentation indicated that most anti-nematode microbial strains are bacteria and *Bacillus* genus. Among the bacteria, previous studies mainly focused on the activity of *Bacillus* genus. Thus, more research and screening are still needed for many other microbial subjects, such as bacteria, fungi, and actinomycetes, to find new and potential natural sources against black pepper RKNs. Besides, most studies only tended to inhibit J2s nematode while anti-hatching of eggs was also supposed to be a pivotal target to control harmful nematodes. Furthermore, the combination of nematode control and plant growth stimulation showed good effects on the management of black pepper nematodes. Thus, besides assessing the ability to inhibit nematodes, investigating the ability to stimulate the growth of microorganisms is also an important strategy in nematode management.

2.2. Effect of active microorganism strains on black pepper seedlings in the greenhouse and the black pepper trees in the field

Several studies were conducted to assess the selected microbial strains' nematocidal and plant-promoting effects on the black pepper seedlings in greenhouse tests in the Central Highlands of Viet Nam [1, 19, 25, 35]. Nguyen et al. assessed the effect of 10 bacterial strains isolated from the rhizome soils of black pepper cultivated in the Central Highlands of Viet Nam [19]. The experimental data of this work demonstrated that these 10 rhizobacterial strains exhibited effective anti-nematode activity and showed high plant growth-promoting effects in the greenhouse, and *Bacillus subtilis* RBDL28 displayed the best effect. The nematode population in the roots and soils was significantly reduced with great inhibition values of 87.17% and 95%, respectively, after three months of treatments with *Bacillus subtilis* RBDL28. In addition, this anti-nematode strain showed positive activity on the growth parameters of black pepper seedlings under greenhouse conditions. In another investigation, Nguyen et al. also screened bacterial strains for managing black pepper and found that *Serratiamarcescens* TNU02

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was the most potent strain with a mortality rate of up to 98.10 %. In greenhouse tests, the nematode population was reduced by up to 70 % (in roots) and 85 % (in soils) by the TNU02 strain [25]. In a work by Tran et al., five active endophytic bacterial strains were tested for their nematocidal and plant-promoting effect under greenhouse conditions [1]. B. megaterium DS9 significantly reduced nematodes in the soil and pepper plant roots with inhibitory values of 81.86 % and 73.11 %, respectively. The strain DS9 showed a significant effect on plant growth promotion, such as the increased shoot length (29.81 cm), formation of new leaves (3.99 leaves), and increased root length (8.98 cm). These values were significantly higher than those of the untreated group, with less increase in shoot length (17.25 cm), less new leaf formation (1.00 leaves), and less increase in root length (5.56 cm). The contents of chlorophyll a+b also showed a higher rate than those of the control group. Recently, Trinh et al. reported the effect of 6 target antagonistic rhizobacterial strains against nematodes using Vinh Linh black pepper seedlings as plant models [35]. The results indicated that all these strains reduced the density of nematodes in roots and soils and demonstrated a positive effect on plant-promoting activity in the greenhouse. Of these, the rhizobacterium Bacillus velezensis EK7 was the most active strain with a potent nematocidal effect. EK7 reduced more than 64 % and 55 % of nematode populations in roots and rhizome soil, respectively, and showed good plant growth-promoting effects. For searching the potential use of beneficial microbes, several bacterial strains were examined under field conditions to explore their nematode-killing and plant growth-promoting effects on the black pepper trees in the fields in the Central Highlands of Viet Nam [19, 36]. Nguyen et al. assessed the efficacy of some beneficial bacterial strains on disease resistance and growth stimulation of black pepper under field conditions in Cu Kuin (Dak Lak, Viet Nam), Dak Mil (Dak Nong, Viet Nam), and Duc Co (Gia Lai, Viet Nam). The result of this work indicated that the combined use of some Bacillus strains, including B. velezensis KN12, B. amyloliquefaciens DL1, B. velezensis DS29, B. subtilis BH15, B. thuringiensis DS8, B. megaterium DS9, B. subtilis RBDL28, B. pumilus RBDS13, B. subtilis V1.21, and B. cereus CS30 significantly reduced nematodes in the pepper plant roots and in the soil after 6 months of treatment with inhibitory values in the range of 33.7 - 85.5 % and 43.9 - 92.62 %, respectively [19]. The formulation containing B. velezensis KN12, B. amyloliquefaciens DL1, B. velezensis DS29, B. subtilis BH15, B. subtilis V1.21, and B. cereus CS30 also demonstrated a high inhibitory activity against some harmful fungi in soil and root of black pepper [36]. Some fungal strains were also isolated from the soil samples in Gia Lai and Dak Lak provinces, Viet Nam, for testing the ability to trap nematodes and their impact on black pepper plants through in vitro analysis, under greenhouse and field conditions [37]. The fungi strains were identified as Arthrobotrys sp. All isolated strains presented a good trap ability on Meloidogyne incognita nematode. Moreover, these fungi reduced the density of nematodes in soils by 53.04 - 62.07 % in greenhouse plots and 61.45 - 69.38 % under field conditions. Besides, the rate of diseased plants decreased by 54.5 - 67.9 % in greenhouse experiments and 26.8 - 54.4 % in field conditions when treated with selected Arthrobotrys nematophagous fungi [37].

Several researches on the anti-RKN activity against black pepper under greenhouse and field conditions have been reported. The biological effect of Bacillus subtilis, Pseudomonas fluorescens, and Trichoderma viridi for black pepper RKNs inhibition was evaluated [38]. The results indicated that all the biological agents could potentially promote plant growth criteria, such as the number of leaves and plant biomass. The maximum number of leaves (40 %) was recorded in plot treatment with Pseudomonas fluorescens, and the highest plant biomass (50 %) was obtained in Pseudomonas fluorescens treatment. Furthermore, these agents reduced significantly the population of nematodes in soil and root compared to the control. Among them, Pseudomonas fluorescens showed the best effect with the reduction rate in nematode population.
in the soil, egg mass/g, and adult female nematode/g reaching the maximum of 60.1 %, 41.2 %, and 38.1 %, respectively [38]. Munif et al. tested the strategy of improving black pepper quality and reducing production cost by using the formulation of endophytic bacteria against Meloidogyne spp. and evaluating its effect on black pepper plant growth under greenhouse and field conditions [39]. In greenhouse experiments, dry formulation of Bacillus sp. AA2 reduced the number of nematodes in soil and increased the number of leaves and branches of black pepper. The result showed a reduction in the incidence of yellowing leaf disease, an improvement in productivity via an increase in the number of flowers, and a reduced number of nematodes in the soil [39]. Munif et al. indicated the role of the combination of endophytic bacteria with organic materials for nematode control in pepper [40]. The results showed that it could stimulate pepper growth and reduced 70 - 90 % of root galls caused by Meloidogyne incognita [40]. In a report by Aravind et al., various endophytic bacteria groups, including Arthrobacter spp. (20 strains), Micrococcus spp. (10 strains), Bacillus spp. (32 strains), Curtobacterium spp. (one strain), Pseudomonads (26 strains), Serratia (one strain), and 20 unidentified strains were used for evaluating the inhibition of black pepper nematodes in the nursery [41]. All groups effectively inhibited Meloidogyne incognita and Radopholus similis nematodes. For in vivo treatment, consortia 1 and 4 significantly reduced the number of nematodes in soil and plant tissues, and they were considered as potential agents for nematode biological control [41]. The efficiency of Paecilomyces lilacinus in the inhibition of root-knot nematodes (Meloidogyne incognita) and burrowing nematodes (Radopholus similis) harming black pepper was studied. This fungus suppressed significantly nematode infestation and increased total root mass production. Besides, it was more effective in suppressing Meloidogyne incognita than R. similis [42]. The ability of Pasteuria penetrans bacteria and Paecilomyces lilacinus fungi to control harmful Meloidogyne incognita on black pepper was studied under greenhouse conditions [43]. These microorganisms reduced the nematode population and significantly improved the growth of the plants and root mass production. In addition, the combination of both organisms was demonstrated to be more effective in managing RKNs in black pepper than using each organism individually. Moreover, both Paecilomyces lilacinus and Pasteuria penetrans did not cause any negative effect on plant growth, and they were still alive and effective even two years later under greenhouse conditions [43]. Two fungal bioagents (Trichoderma harzianum and Pochonia chlamydospora) and an endophytic bacteria (Pasteuria penetrans) were assessed for the management of black pepper nematodes under field conditions within 5 years (1998 - 2001) in India. A reduction in yellowing leaf phenomena was noticed after the first year of treatment. The lowest incidence of yellowing after four years was achieved when treated with Trichoderma harzianumby (15.25 %), followed by plots treated with Pochonia chlamydospora (20.78 %) and the final was Pasteuria penetrans (24.13 %). However, the highest productivity (1.83 kg vine-1) was obtained in Pochonia chlamydospora treated plots. The lowest nematode level was recorded in the Pochonia Chlamydospora treatment [44]. Koshy et al. indicated the role of using individually or in combinations with bio-agents, including Arbuscular mycorrhizal, Paecilomyces lilacinus, and Pasteuria penetrans, for the management of R. similis infestation black pepper plant under field conditions within four years [45]. Stem and root bacterization of black pepper before planting were recorded as a unique strategy to protect the plants from pathogens and enhance the growth of rooted cuttings [46]. Bacterialization of tree stumps with Curtobacterium luteum and Bacillus megaterium reduced over 70 % of nematodes in soil and created over 65 % of nematode-free plantlets [46]. Some bacteria were isolated from black pepper to suppress R. similis [47]. Through preliminary screening by invitro and invivo tests, six selected strains showed a good anti-nematode effect,
Among them, two of the endophytic bacteria identified as *Bacillus megaterium* and *Curtobacterium luteum* were promising candidates for the management of *R. similis* [47].

Overall, many potential microbial strains resistant to pepper nematodes were tested under greenhouse and field conditions with varying potential results. These results indicated that microorganisms are promising candidates for the eco-friendly management of RKNs infected on black pepper and also open up new application directions for these microorganisms. There have been numerous works assessing the antinematode effect of various beneficial microbial strains under greenhouse conditions and showing promising results in the Central Highlands of Vietnam; however, studies assessing under field conditions are still limited. Thus, further studies are needed to comprehensively examine and evaluate the effects of microbial strains on nematodes, pepper plants, soil, and beneficial soil microbial under field conditions.

### 3. BIO-PRODUCTION AND APPLICATION OF ANTIMICROBIAL COMPOUNDS FOR MANAGEMENT OF BLACK PEPPER ROOT-KNOT NEMATODES

#### 3.1. Nematocidal compounds from microorganisms

The biological controls of RKNs are mainly related to antagonism against RKNs or promoting plant growth to enhance its health to protect it from the harmful impact of nematodes [48]. Although a series of active compounds from various sources such as synthetic chemistry, plant extracts, and microorganisms were discovered for RKNs inhibition, secondary metabolites from bacteria and fungi were considered prominent antagonists for the biocontrol of RKNs [48, 49]. Some natural bioactive compound groups on antagonism RKNs are well known, including phenols, aldehydes, ketones, acids, esters, thiocyanates, macrolides, amides, alkaloids, terpenoids, and peptides [50]. Table 2 summarises secondary metabolites from bacteria, actinomycyes, and fungi in the inhibition of harmful RKNs in agriculture. 2-octanone collected from *Pseudomonas putida* was anti- *Meloidogyne incognita* with an IC$_{50}$ value of 22.7 mg/L after 48 h; 2-octanone at concentrations from 1000 to 10000 mg/L, was recorded to exhibit a repellent effect on this nematode [51]. The activity of fervenulin from *Streptomyces* sp. against *Meloidogyne incognita* after 96 h reached up to 100 % at 250 mg/L, and it was non-effective against nematode eggs [52]. Spectinabinin from *Streptomyces* spp. showed a significant effect against *Bursaphelenchus xylophilus* (a pine wood nematode) with an IC$_{50}$ value of 0.84 mg/L, and it effectively inhibited the pine wilt disease at a concentration of 0.9 mg/plant under greenhouse conditions [53]. The macrolide compound was purified from the fermentation broth of *Streptomyces avermitilis* TM24 mutant strain, and it inhibited the activity of *Bursaphelenchus xylophilus*, showing an LC$_{50}$ value of 4.3 mg/L [54]. The compounds from *Bacillus* spp. against *Bursaphelenchus xylophilus* were examined, and they showed significant effect with IC$_{50}$ values from 232 to 904 ppm [55]. The activity of fungi chromin B from *Streptomyces albogriseolus* against knot-root nematodes, including *Meloidogyne incognita* and *M. javanica*, was tested showing a good effect with IC$_{50}$ values of 7.64 and 7.83 μg/mL, respectively [56]. A linear peptide compound, rhabdopeptide from the fermentation broth of *Xenorhabdus budapestensis*, presented nematicide inhibitory activity against *Meloidogyne incognita* with an IC$_{50}$ value of 27.8 mg/L [57]. Kojic acid isolated from *Aspergillus oryzae* fungi presented the activity against *Meloidogyne incognita* J2s and eggs, with IC$_{50}$ values of 195.2 mg/L and 238.3 mg/L, respectively [58].

The secondary metabolites produced by the fungal endophytic *Fusarium oxysporum* elucidated inhibitory activity against *Meloidogyne incognita*. Indole-3-acetic acid, 4-
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hydroxybenzoic acid, and gibeyrone D showed an intense anti-nematode activity after 72 h of treatment with IC\textsubscript{50} values of 117, 104, and 134 μg/mL, respectively [59]. 3-methylbutyl acetate from \textit{F. oxysporum} showed activity against \textit{Meloidogyne incognita} J2s with an IC\textsubscript{50} value of 198 mg/L, and it inhibited around 90 % of egg-hatching [60]. Alternariol 9-methyl ether from Alternaria spp. inhibited \textit{Bursaphelenchus xylophilus} with an IC\textsubscript{50} value of 98.17 μg/mL [61]. α,β-dehydrocurvularin from \textit{Aspergillus welwitschiae} inhibited nematode infestation (\textit{Meloidogyne graminicola}) on rice roots, with an IC\textsubscript{50} value of 122.2 mg/L. Furthermore, it inhibited nematode growth and reduced root-knot index under greenhouse conditions [62]. Thermolides from \textit{Talaromyces thermophilus} fungus presented a potential activity against \textit{Meloidogyne incognita} with an IC\textsubscript{50} value of 0.7 - 55.6 μg/mL [63]. The compounds from \textit{Myrothecium verrucaria} fungus, including verrucarin A and roridin A inhibited \textit{Meloidogyne incognita} with IC\textsubscript{50} values of 1.8 and 1.5 mg/L, respectively [64]. Gymnoascole acetate from \textit{Gymnoascus reessii} fungus demonstrated a potent effect on \textit{Meloidogyne incognita} J2s with an IC\textsubscript{50} value of 47.5 μg/mL, and it also exhibited a high inhibition of egg-hatching (nearly 90 %) [65].

\textit{Table 2. List of compounds from fungi and bacterials inhibiting harmful root-knot nematodes in agriculture.}

<table>
<thead>
<tr>
<th>Compound</th>
<th>Microorganism</th>
<th>Nematode</th>
<th>Anti-RKN activity</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Anti-J2s</td>
<td>Anti-eggs</td>
</tr>
<tr>
<td><strong>Bacteria, Actinomyces</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-octanone</td>
<td>\textit{Pseudomonas putida}</td>
<td>\textit{M. incognita}</td>
<td>22.7 mg/L</td>
<td>ND</td>
</tr>
<tr>
<td>Fervenulin</td>
<td>\textit{Streptomyces sp.}</td>
<td>\textit{M. incognita}</td>
<td>100 %</td>
<td>ND</td>
</tr>
<tr>
<td>Spectinabilin</td>
<td>\textit{Streptomyces sp.}</td>
<td>\textit{B. xylophilus}</td>
<td>0.84 mg/L</td>
<td>ND</td>
</tr>
<tr>
<td>Macrolide</td>
<td>\textit{S. avermitilis}</td>
<td>\textit{B. xylophilus}</td>
<td>4.3 mg/L</td>
<td>ND</td>
</tr>
<tr>
<td>4-oxabicyclo[3.2.2]nona-1(7), 5,8-triene</td>
<td>\textit{Bacillus sp.}</td>
<td>\textit{B. xylophilus}</td>
<td>904.1 ppm</td>
<td>ND</td>
</tr>
<tr>
<td>(3S, 8as)-hexahydropyrido[1,2-alpyrazine-1, 4-dione</td>
<td></td>
<td></td>
<td>451.2 ppm</td>
<td>ND</td>
</tr>
<tr>
<td>Phenylacetamide</td>
<td></td>
<td></td>
<td>232.9 ppm</td>
<td>ND</td>
</tr>
<tr>
<td>Fungichromin B</td>
<td>\textit{Streptomyces alboegriseolus}</td>
<td>\textit{M. incognita}</td>
<td>7.64 μg/mL</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td>\textit{M. javanica}</td>
<td>7.83 μg/mL</td>
<td>ND</td>
</tr>
<tr>
<td>Rhabdopeptides</td>
<td>\textit{Xenorhabdus budapestensis}</td>
<td>\textit{M. incognita}</td>
<td>27.8 mg/L</td>
<td>ND</td>
</tr>
<tr>
<td><strong>Fungi</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kojic acid</td>
<td>\textit{Aspergillus oryzae}</td>
<td>\textit{M. incognita}</td>
<td>195.2 mg/L</td>
<td>238.3 mg/L</td>
</tr>
<tr>
<td>Compound</td>
<td>Species</td>
<td>Concentration (µg/mL)</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>-----------------------------------------</td>
<td>----------------------------------</td>
<td>-----------------------</td>
<td>-----------</td>
<td></td>
</tr>
<tr>
<td>Gibepyrone D: E configuration</td>
<td><em>F. oxysporum</em></td>
<td>134.3</td>
<td>[59]</td>
<td></td>
</tr>
<tr>
<td>Gibepyrone G: Z configuration</td>
<td></td>
<td>265.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indole-3-acetic acid</td>
<td></td>
<td>117.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indole-3-acetic acid methyl ester</td>
<td></td>
<td>218.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4-Hydroxybenzoic acid</td>
<td></td>
<td>104.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methyl4-hydroxybenzoate</td>
<td></td>
<td>253.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methyl 2-(4-hydroxyphenyl)aceta te</td>
<td></td>
<td>149.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uridine</td>
<td></td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fusarinolic acid</td>
<td></td>
<td>600.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-(But-3-en-1-yl)picolinic acid</td>
<td></td>
<td>655.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beauvericin</td>
<td></td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carbofuran</td>
<td></td>
<td>64.1 µg/mL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aldicarb</td>
<td></td>
<td>180.7 µg/mL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-Methylbutyl acetate</td>
<td><em>F. oxysporum</em></td>
<td>198 mg/L</td>
<td>[60]</td>
<td></td>
</tr>
<tr>
<td>Alternariol 9-methyl ether</td>
<td><em>Alternaria</em> sp.</td>
<td>74.62 µg/mL</td>
<td>[61]</td>
<td></td>
</tr>
<tr>
<td>A,β-dehydrocurvularin</td>
<td><em>Aspergillus</em> welwitschiae</td>
<td>122.2 mg/L</td>
<td>[62]</td>
<td></td>
</tr>
<tr>
<td>Thermolide A</td>
<td><em>Talaromyces thermophilus</em></td>
<td>0.8 µg/mL</td>
<td>[63]</td>
<td></td>
</tr>
<tr>
<td>Thermolide B</td>
<td><em>M. incognita</em></td>
<td>0.7 µg/mL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thermolide C</td>
<td></td>
<td>30.5 µg/mL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thermolide D</td>
<td></td>
<td>55.6 µg/mL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Verrucarin A</td>
<td><em>Myrothecium verrucaria</em></td>
<td>1.8 mg/L</td>
<td>[64]</td>
<td></td>
</tr>
<tr>
<td>Roridin A</td>
<td></td>
<td>1.5 mg/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gymnoascole acetate</td>
<td><em>Gymnoascus reessii</em></td>
<td>47.5 µg/mL</td>
<td>[65]</td>
<td></td>
</tr>
</tbody>
</table>

In Viet Nam, several studies reported compounds from microorganisms against black pepper nematodes. Based on LC/MS analysis, two main compounds were detected from the fermentation broth of *Bacillus subtilis* RB.DL.28, including ulfacetamide and metronidazole-OH [5]. Hemi-pyocyanin was isolated from *Pseudomonas aeruginosa* TUN03 fermentation and was
used to evaluate inhibitory activity against *Meloidogyne incognita* [66]. This compound showed a significant effect on both nematode J2s and eggs hatching with IC$_{50}$ values of 0.377 μg/mL and 301 μg/mL, respectively [66]. Prodigiosin, a red pigment obtained from *Serratia marcescens* TUN02, was also assessed for the inhibition of black pepper nematodes [14]. The purified pigment presented a potential inhibitory activity on J2s and egg-hatching with IC$_{50}$ values of 0.2 and 0.32 mg/mL, respectively [14]. Besides, the promising activity of prodigiosin might be associated with anti-acetylcholinesterase (AChE) - a common enzyme used to resist *Meloidogyne* nematode [67]. Compounds from *Bacillus veleznesis* RB.EK7 fermentation were identified as thymine and hexahydropyrrolo [1,2-al]pyrazine-1,4-dione [68]. These compounds were tested for anti-*Meloidogyne incognita* effect on black peppers. Thymine showed a significant effect on nematodes with a mortality rate of up to 100 % for J2s and egg-hatching inhibition of 70.1 %. [1,2-al]pyrazine-1,4-dione displayed an anti-J2s activity with a mortality rate value of 64.2 % and inhibited egg-hatching at 57.9 %. These findings indicate that these compounds are promising inhibitors for the AChE enzyme, which is significantly related to the resistance of nematode *Meloidogyne incognita* [68].

Many studies have reported anti-J2s compounds from different microbial sources including bacteria, actinobacteria, and fungi on the inhibition of some kinds of black pepper nematodes such as *M. incognita*, *B. xylophilus*, *M. javanica*, and *M. graminicola*. However, only a few researches evaluated egg-hatching inhibition. The prospective nematicidal candidates are considered to inhibit both J2 nematodes and egg hatching. Thus, more studies on the anti-egg-hatching effect of bioactive compounds are recommended. Furthermore, because the exploration of anti-nematode mechanisms is limited, with only a few reports conducted via virtual screening. Thus, more research is needed to resolve this aspect in the future.

### 3.2. Biotechnology for eco-friendly production of nematocidal compounds

Though various nematocidal compounds have been discovered, several works have been reported concerning an eco-friendly and scale-up production of potential nematocidal compounds. In a report by Trinh et al., some organic wastes were used to produce nematicidal compounds from *Bacillus veleznesis* RB.EK7 [68]. Shrimp shell powder was demonstrated to be the most suitable substrate for creating anti-nematode compounds. However, this study only conducted fermentation on a small scale (in a flask) [68]. In other studies by Nguyen et al., phenazine compounds were found to be good nematicidal inhibitors [66, 69]. The production of these potential compounds was scaled up on a large scale (bioreactor systems) using by-products as the main substrate. This fermentation process showed that it was cost-effective and environmentally friendly; furthermore, it took shortened cultivation time and showed a higher nematicidal compound yield [66, 69]. A red pigment, prodigiosin (PG), was also considered a promising anti-nematode inhibitor with strong effects on both objects of black pepper nematodes (juveniles and eggs) [14]. This compound was produced in a bioreactor system in Viet Nam and used in various studies based on eco-friendly approaches (Table 3 summarises the related reports). Most of the reports noted effective fermentation time with shortening from 2 - 3 days to 8 - 10 h. Some agro-byproducts were utilized for PG production, such as peanut oil cake [14], soybean residue by-product [70], and cassava wastewater [71], and in these media, high PG yields in the range of 5700 to 6886 mg/L were obtained. Various marine by-products rich in chitin sources, including crab shells [72], squid pens [73], shrimp shells [74], and shrimp heads [75], were also reused for PG biosynthesis, with PG productivity reaching from 3450 to 6310 mg/L.
Table 3. Some reports on the production of nematicidal compounds in bioreactor systems.

<table>
<thead>
<tr>
<th>Bacteria producer</th>
<th>Main substrate</th>
<th>Fermentation time (hours)</th>
<th>Yield (mg/L)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Production of phenazine compounds</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>P. aeruginosa</em> TUN03</td>
<td>Squid pens</td>
<td>12</td>
<td>22.73</td>
<td>[66]</td>
</tr>
<tr>
<td><strong>Production of prodigiosin</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. marcescens</em> TUN02</td>
<td>Peanut oil cake</td>
<td>10</td>
<td>6886</td>
<td>[14]</td>
</tr>
<tr>
<td><em>S. marcescens</em> TUN02</td>
<td>Soybean residue by-product</td>
<td>10</td>
<td>5700</td>
<td>[70]</td>
</tr>
<tr>
<td><em>S. marcescens</em> TUN02</td>
<td>Demineralized crab shells</td>
<td>8</td>
<td>5100</td>
<td>[72]</td>
</tr>
<tr>
<td><em>S. marcescens</em> TNU01</td>
<td>Squid pens</td>
<td>12</td>
<td>3450</td>
<td>[73]</td>
</tr>
<tr>
<td><em>S. marcescens</em> TUN02</td>
<td>Demineralized shrimp shells</td>
<td>8</td>
<td>6200</td>
<td>[74]</td>
</tr>
<tr>
<td><em>S. marcescens</em> CC17</td>
<td>Shrimp heads</td>
<td>8</td>
<td>6310</td>
<td>[75]</td>
</tr>
<tr>
<td><em>S. marcescens</em> TNU01</td>
<td>Cassava wastewater</td>
<td>8</td>
<td>6150</td>
<td>[71]</td>
</tr>
</tbody>
</table>

Overall, recent advances in the eco-friendly and scaling-up bioproduction of anti-nematode inhibitors via microbial fermentation have facilitated reductions in cost and fermentation time, while also considering environmental issues. Although this strategy showed a good effect on the production of nematicidal agents, there have been only a few related reports. So, this research orientation needs to be conducted on a large-scale and on an industrial scale towards the application of microbial compounds in the management of black pepper nematodes in the near future.

4. CONCLUSIONS AND PERSPECTIVES

This overview summarised the management strategy of black pepper nematodes in *invitro*, greenhouse, and field conditions using antagonistic microorganisms or their secondary metabolites. The eco-friendly production of nematicidal inhibitors was also discussed. Several rhizomicrobes and endophytic microbes have shown good effects on the inhibition of black pepper nematodes in the Central Highlands of Viet Nam in *invitro* conditions. Moreover, some strains demonstrated their potential effects under greenhouse or field conditions. The use of different by-product sources for fermentation is a new trend in the production of nematicidal inhibitors on a large scale in a cost-effective and environmentally friendly manner, in which higher compound productivity and shortened fermentation time were obtained. In general, exploiting the potential of microorganisms and their compounds in the management of black pepper nematodes is a direction with many advantages in terms of activity and production in practice.

In Viet Nam, various microbes possessing the anti-nematode ability to infest black pepper have been reported, while other agents, such as fungi and actinomycetes, have not yet been noticed. Studies on the inhibitory activity have mainly focused on the inhibition of second-stage juveniles, while the understanding of the effects on egg-hatching is still limited. Furthermore, most of the studies have mainly focused on *invitro* tests, or extensive trials to test under greenhouse conditions, while only a few field experiments have been reported. Other aspects of research to control black pepper nematodes in Viet Nam, such as the isolation of nematicidal
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compounds and their mechanisms for inhibition of nematodes, are still left untapped. Especially, only a few studies targeting the production of potential anti-nematode compounds have been reported. Moreover, works concerning the effect of beneficial microbes and their active metabolites on soil properties as well as soil microbes have been reported very little. Therefore, in the coming time, all the above-mentioned issues should be paid more attention to, towards the production of pepper in a safe, efficient, and sustainable manner.

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Conflicts of interest: The authors declare no conflict of interest.

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