The Andhra Agric. J 71 (1): 047-053, 2024
doi :10.61657/aaj.2024.91

Antagonistic potential of plant growth promoting rhizobacteria against *Phytophthora palmivora* in Cocoa

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ABSTRACT

Pod rot disease in cocoa (*Theobroma cacao* L), caused by *Phytophthora palmivora* (Butler) has been identified as one of the main production constraints in all cocoa growing areas across the world. *P. palmivora* (Butler) is a hemibiotrophic oomycete capable of infect over 200 plant species. To manage the pod rot disease, plant growth promoting rhizobacteria plays an important role. In this study we screened the 50 bacterial isolates against the *Phytophthora palmivora* under *in vitro*, among the 50 bacterial isolates tested, NEG27 isolate was found significantly superior in its radial growth (1.60 cm) with maximum inhibition per cent (82.26%). This was followed by NWG12 with 2.03 cm radial growth and inhibition per cent of 77.41% which was on par with NEG14 (2.13 cm) with mycelial inhibition of 76.30%. Continued incubation up to four DAI indicated that only nine bacterial isolates NEG27 (3.40), NWG16 (2.97), NEG14 (2.87), NEG9 (2.32), NWG12 (2.03), NSK3 (1.40), NWG14 (1.20), NEG3 (0.50), NEG6 (0.36) produced characteristic Zone of inhibition against *P. palmivora.*

Keywords: *Bacterial isolates, Cocoa, Pod rot and P. palmivora*

Cocoa (*Theobroma cacao* L.) is an important commercial plantation crop grown in the humid tropics. In India, it is mainly cultivated in Andhra Pradesh, Karnataka, Kerala and Tamil Nadu, often as an intercrop with oilpalm and coconut. The area under cocoa cultivation is gradually expanding due to promotion by chocolate-producing companies through contract farming. Cocoa trees are small evergreen plants, reaching heights of 4 to 8 meters. The dried and fully fermented fatty seeds of the cacao tree yield cocoa, which serves as the basis for making chocolate. Cocoa can be grown up to 300 meters above mean sea level and requires a minimum of 90-100 mm rainfall per month, with an annual rainfall of 1500-2000 mm. Well-drained soils with a pH range of 6.5 to 7.0 are ideal for cocoa cultivation. Shade is essential for cocoa, and it thrives in plantations where approximately 50% of light is available. Coconut and oilpalm gardens are well suited for cultivating cocoa.

Though cocoa being raised as intercrop with coconut and oil palm especially in Andhra Pradesh, its area has increased considerably from 24,156 ha (2016-2017) to 36, 455 ha (2019-2020) due to increased industrial importance of cocoa (dccd.gov.in). Several important fungal diseases and pests have gained prominence in the last two decades and pose a serious threat to the supply of chocolate. One among the fungal disease is pod rot disease caused by *Phytophthora palmivora.*

Cacao black pod rot is caused by a pathogen in the genus *Phytophthora*, which means as "plant destroyer." This is the genus that caused the Irish potato famine of 1845-1852. These pathogens were originally classified as fungi, but they have since been reclassified as Stramenopila. There are over 80 Phytophthora species that cause plant diseases, with several, including *P. palmivora, P. megakarya, P. citrophora, and P. capsici,* being responsible for cacao black pod rot. *Phytophthora* spp. is responsible for 20 to 30% of the total cacao crop loss each year, with some plantations losing up to 90% of their pods due to the disease (Acebo-Guerrero *et al*., 2012).

Black pod rot disease caused by *Phytophthora palmivora* is a devastating disease affecting cocoa (*Theobroma cacao* L.) plantations worldwide. This hemibiotrophic oomycete can infect over 200 plant species, including cocoa. It targets various parts of the cocoa plant, such as the main stems, jarquets, cushions, and pods, leading to black pod rot and stem canker disease. The disease spreads through infected plant debris, soil, and water. Pod rot causes significant yield losses, with estimates suggesting up to 30% reduction in total crop production, translating to substantial economic losses for cocoa producers globally (Delgadillo- Duran *et al*., 2020).

MATERIAL AND METHODS

A total of 50 bacteria were isolated from cocoa rhizoplane and rhizosphere samples from different cropping systems especially coconut/oilpalm cropping sytems which are collected from major cocoa growing districts *viz*., East Godavari, West Godavari and Srikakulam.

To assess the antagonistic ability of 50 rhizobacterial isolates, they were screened against cocoa pod rot pathogen *P. palmivora* by employing the dual culture technique (Dennis and Webster, 1971).

Sterilized potato dextrose agar medium (without any antibiotic) was poured aseptically into sterile Petri plates under laminar air flow chamber and allowed for solidification. Each plate was inoculated in the centre with a 5 mm diameter disc was cut by corkborer from the periphery of actively growing four day old *P. palmivora* with the help of an inoculation loop, test bacterial isolate from a 48 to 72 h old actively growing culture was streaked parallely on both sides of fungal disc in such a way that it is 2.5 cm away from the fungal disc, while plates inoculated with *P. palmivora* alone served as control and the plates were incubated at 25±2 ºC for four days, and replicated thrice. At the end of incubation, the colony diameter of *P. palmivora* was measured and the per cent inhibition of *P. palmivora* was calculated by adopting the following formula given by Vincent (1927)

$$I=(C-T / C)\times 100$$

Where.

I = Per cent inhibition over control

C = Growth of the pathogen in control plate

T = Growth of the pathogen in dual cultured plate

Statistical analysis

Data on radial growth of *P. palmivora* was recorded and calculated the per cent inhibition over control at 1st, 2nd, 3rd and Fourth days after inoculation and the data was subjected to the Arcsine transformation (arcsine) and further analysed by completely randomized block design. The data analysis was done in MS excel by using data analysis tool packs.

RESULTS AND DISCUSSION

Dual culture technique (Dennis and Webster, 1971) was used for rapid screening of Plant growth promoting rhizobacteria (PGPR) bacterial isolates for their antagonistic potential against *P. palmivora*. It offers one to one interaction of pathogen and antagonist, where in, the competition exists for nutrients and space.

A total of 50 PGPR bacterial isolates were screened for their antagonistic efficacy against *P. palmivora.* Wide variation existed in the pathogen growth when dual cultured with different PGPR isolates. The radial growth (cm) of *P. palmivora* in dual cultured plates ranged from 1.60 (NEG27) to 9.00 (NEG19, NWG 4 and NSK 2) at four days after inoculation (Table 4.9 and Plate 4.14 and 4.15). All PGPR bacterial isolates except NEG19, NWG4 and NSK2 had inhibited pathogen with an inhibition ranging from 0.37 (NEG11) to 82.26 (NEG 27) per cent inhibition over control after four days of inoculation (Table 4.1 and Fig. 4.1). In control (monoculture), *P. palmivora* radial growth was 2.50, 7.17, 8.50 and 9.00 cm after first, second, third and fourth day of inoculation respectively.

In the present investigation, zone of Inhibition (ZI) between the *P. palmivora* and test bacterial isolates after first DAI revealed the range from 2.30 (NEG13, NWG 4 and NWG 16) to 4.30 (NEG 27) while per cent inhibition of *P. palmivora* after one DAI varied between 8.00 (NEG13, NWG 4, NWG 16) to 72.00 (NEG27).

Even after two DAI, continued per cent inhibition over control in the growth of *P. palmivora* was observed and it ranged from 24.66 (NWG4) to 38.13% (NWG 12), indicating that the diffusible metabolites secreted by PGPR isolates may be responsible for inhibiting the growth of *P. palmivora* which was evident by the presence of ZI in each interaction and it ranged from 4.32 (NEG27) to 5.53 cm (NEG2)

This indicates that the PGPR isolates produce metabolites that actively inhibit the growth of *P. palmivora*, demonstrated their potential as biological control agents. The variations in inhibition percentages and ZI sizes in cm among different isolates might be due to differences in the types and concentrations of metabolites produced by each PGPR bacterial isolate. Among the nine potential PGPR bacterial isolates (NEG3, NEG6, NEG9, NEG14 NEG16, NEG27, NWG12, NWG14 and NSK3) that performed well in dual culture.

After four DAI, among the 50 bacterial isolates tested, NEG27 isolate was found significantly superior in its radial growth (1.60 cm) with maximum inhibition per cent (82.26%). This was followed by NWG12 with 2.03 cm radial growth and inhibition per cent of 77.41% which was on par with NEG14 (2.13 cm) with mycelial inhibition of 76.30%. Continued incubation up to four DAI indicated that only nine bacterial isolates NEG27 (3.40), NWG16 (2.97), NEG14 (2.87), NEG9 (2.32), NWG12 (2.03), NSK3 (1.40), NWG14 (1.20), NEG3 (0.50), NEG6 (0.36) produced characteristic ZI against *P. palmivora*. (Table 4.1 and Fig. 4.1) indicating strong antagonistic activity limiting the growth of the *P. palmivora.* Results indicated their strong antagonistic properties and differences in inhibition levels among the PGPR bacterial isolates are likely due to variations in the types and concentrations of the antifungal compounds produced, as well as the competitive interactions for nutrients and space in the culture medium.

Melnick *et al.* (2008) reported that some *Bacillus* spp. from cocoa were capable of long-term colonization of cocoa leaves and subsequent disease reduction of pod rot. They inhibited *P. palmivora* mycelial growth in the range of 7 to 57 per cent. Larbi-Koranteng *et al*. (2020) tested the efficacy of cocoa rhizobacterial isolates against *P. palmivora* and found that *B. amyloliquefaciens* ESI to inhibit *P. palmivora* with the highest inhibition zone of 21.21 mm.

Similarly, Thomas *et al*. (2011) reported that of 519 isolates obtained from the rhizosphere and roots of cocoa trees from different locations in South India, 359 were *Bacillus* spp., where 44 were rhizospheric and 45 were endophytic while 160 were fluorescent *Pseudomonas* spp., that were found to be antagonistic to *P. palmivora*. They reported that four *Bacillus* spp., (one from Pollachi - *Bacillus* sp. PSB6 and three from Kasaragod - *Bacillus* sp. KGSB5, *Bacillus* sp. KGSB11 and *Bacillus* sp.

in South India, 359 were *Bacillus* spp., where 44 were rhizospheric and 45 were endophytic while 160 were fluorescent *Pseudomonas* spp., that were found to be antagonistic to *P. palmivora*. They reported that four *Bacillus* spp., (one from Pollachi - *Bacillus* sp. PSB6 and three from Kasaragod - *Bacillus* sp. KGSB5, *Bacillus* sp. KGSB11 and *Bacillus* sp.
KGSB26) resulted in reduction of 57 % mycelial growth of the fungal pathogen. *Bacillus* spp. isolated from Tamil Nadu were reported with higher antagonistic potential (48% of rhizospheric and 76% of endophytic *Bacillus* spp.) than bacterial isolates obtained from other states. *P. fluorescence* and *B. subtilis* were reported to inhibit the fungal growth of *P. palmivora* isolated from cocoa pod rot samples under *in vitro* conditions (Pratama *et al*., 2013). This inhibition of pathogen growth by PGPR may be due to the production of diffusible metabolites like antibiotics and may also due to production cell wall degrading enzymes which lead to the fungal inhibition.

Similarly, Acebo Guerrero *et al*. (2015) tested 127 rhizobacteria isolated from cacao rhizosphere and identified three isolates CP07, CP24 and CP30 as *Pseudomonas chlororaphis*, which showed *in vitro* antagonistic activity against *P. palmivora*.

Sharifuddin (2000) identified nine potential antagonistic bacteria against *P. palmivora* and *P. nicotianae* from cocoa rhizosphere. Of the 103 endophytic bacterial isolates that were isolated from healthy cocoa tissues (leaves, branches and fruits) from seven states of Malaysia (Alsultan *et al*., 2019) two isolates AS1 and AS2 were stated with more than 80% inhibition of *P. palmivora* radial growth. Similarly, of the 106 isolates obtained from three cacao progenies only 17 were reported to be potential antagonistic bacterial isolates against *Phytophthora palmivora* (Akrofi *et al*., 2017). Out of 48 bacterial isolates more than 65% inhibition against all tested *Phytophthora* species (Syed-Ab-Rahman *et al*., 2018).

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Table 4.1. Antagonistic efficacy of test bacterial isolates on radial growth of *P. palmivora* in dual culture under *in vitro*

S. No.	*P. palmivora* dual cultured with bacterial isolates	1 DAI			2 DAI			3 DAI			4 DAI		
		Radial growth (cm)	Inhibition (%)	ZI (cm)	Radial growth (cm)	Inhibition (%)	ZI (cm)	Radial growth (cm)	Inhibition (%)	ZI (cm)	Radial growth (cm)	Inhibition (%)	ZI (cm)
1	NEG1	1.8	28.00 (31.95)	3.2	4.49	37.39 (37.69)	0.51	5.27	38.04 (37.69)	-	7.5	16.67 (24.09)	-
2	NEG2	1.32	47.20 (43.39)	3.68	5.53	22.78 (28.50)	-	5.53	34.90 (28.50)	-	6.53	27.41 (31.57)	-
3	NEG3	2.2	12.00 (20.27)	2.8	4.7	34.36 (35.88)	0.3	4.9	42.35 (35.88)	0.1	2.68	68.59 (55.94)	2.32
4	NEG4	1.9	24.00 (29.33)	3.1	4.77	33.48 (35.35)	0.23	5.03	40.78 (35.35)	-	6.13	31.85 (34.36)	-
5	NEG5	2.17	13.33 (21.37)	2.83	5.23	26.97 (31.28)	-	5.23	38.43 (31.28)	-	5.5	38.89 (38.58)	-
6	NEG6	1.8	28.00 (31.95)	3.2	4.52	36.97 (37.45)	0.48	4.52	46.86 (37.44)	0.48	4.64	48.44 (44.11)	0.36
7	NEG7	2.1	16.00 (23.56)	2.9	5.45	23.99 (29.31)	-	5.45	35.92 (29.31)	-	5.62	37.52 (37.77)	-
8	NEG8	2	20.00 (26.57)	3	5	30.23 (33.35)	-	5.27	38.04 (33.35)	-	5.5	38.89 (38.58)	-
9	NEG9	1.2	52.00 (46.15)	3.8	4.5	37.21 (37.59)	0.5	4.5	47.06 (37.59)	0.5	4.5	50.00 (45.00)	0.5
10	NEG10	2.2	12.00 (20.27)	2.8	5.23	26.97 (31.28)	-	5.23	38.43 (31.28)	-	5.23	41.85 (40.31)	-
11	NEG11	2.2	12.00 (20.27)	2.8	4.75	33.76 (35.52)	0.25	6.1	28.24 (35.52)	-	8.97	0.37 (2.02)	-
12	NEG12	1.5	40.00 (39.23)	3.5	5.13	28.36 (32.18)	-	5.5	35.29 (32.18)	-	6.5	27.78 (31.81)	-
13	NEG13	2.3	8.00 (16.43)	2.7	4.87	32.09 (34.50)	0.13	5.2	38.82 (34.50)	-	5.5	38.89 (38.58)	-
14	NEG14	1.5	40.00 (39.23)	3.5	4.67	34.88 (36.20)	0.33	4.67	45.10 (36.19)	0.33	2.13	76.30 (60.87)	2.87
15	NEG15	2.2	12.00 (20.27)	2.8	4.8	33.02 (35.07)	0.2	5.5	35.29 (35.07)	-	6.2	31.11 (33.90)	-
16	NEG16	1.4	44.00 (41.54)	3.6	4.83	32.56 (34.79)	0.17	4.83	43.14 (34.79)	0.17	2.97	43.70 (41.38)	
17	NEG17	2.1	16.00 (23.58)	2.9	5.33	25.58 (30.37)	-	7.25	14.71 (30.37)	-	8.5	5.56 (13.63)	-
18	NEG18	1.93	22.67 (28.36)	3.07	4.51	37.08 (37.51)	0.49	5.35	37.06 (37.51)	-	7.5	16.67 (24.09)	-
19	NEG19	1.77	29.33 (32.78)	3.23	5.23	26.98 (31.29)	-	7.2	15.29 (31.28)	-	9	0.00 (0.00)	-
20	NEG20	1.93	22.67 (28.36)	3.07	4.92	31.35 (34.05)	0.08	5.5	35.29 (34.05)	-	6.5	27.78 (31.81)	-
21	NEG21	2	20.00 (26.57)	3	5.1	28.83 (32.48)	-	5.8	31.76 (32.48)	-	7	22.22 (28.13)	-
22	NEG22	1.93	22.67 (28.36)	3.07	4.85	32.37 (34.67)	0.15	6.5	23.53 (34.67)	-	7.6	15.56 (23.23)	-
23	NEG23	1.9	24.00 (29.33)	3.1	5.5	23.25 (28.83)	-	5.83	31.37 (28.83)	-	6.2	31.11 (33.90)	-
24	NEG24	2	20.00 (26.57)	3	5.23	26.98 (31.29)	-	6.3	25.88 (31.29)	-	7.23	19.63 (26.30)	-
25	NEG25	1.5	40.00 (39.23)	3.5	4.63	35.39 (36.51)	0.37	5.5	35.29 (36.51)	-	6.5	27.78 (31.81)	-
26	NEG26	1.97	21.33 (27.49)	3.03	5.17	27.91 (31.89)	-	5.17	39.22 (31.89)	-	5.17	42.59 (40.47)	-

27	NEG27	0.7	72.00 (58.05)	4.3	4.32	39.72 (39.07)	0.68	4.32	49.18 (39.06)	0.68	1.6	82.26 (65.09)	3.4
28	NWG1	2	20.00 (26.57)	3	4.53	36.74 (37.31)	0.47	5.9	30.59 (37.31)	-	7.27	19.26 (26.03)	-
29	NWG2	2.2	12.00 (20.09)	2.8	5.03	29.77 (33.06)	-	5.5	35.29 (33.06)	-	6.4	28.89 (32.51)	-
30	NWG3	2.1	16.00 (23.58)	2.9	4.73	33.95 (35.64)	0.27	5.03	40.78 (35.64)	-	6.23	30.74 (33.67)	-
31	NWG4	2.3	8.00 (16.43)	2.7	5.4	24.66 (29.77)	-	6.03	29.02 (29.77)	-	9	0.00 (0.00)	-
32	NWG5	2.23	10.67 (18.99)	2.77	5	30.23 (33.35)	-	5.5	35.29 (33.35)	-	5.5	38.89 (38.58)	-
33	NWG6	2	20.00 (26.57)	3	5	30.23 (33.35)	-	5.83	31.37 (33.35)	-	6.43	28.52 (32.27)	-
34	NWG7	2	20.00 (26.57)	3	5.2	27.44 (31.59)	-	7.27	14.51 (31.59)	-	8.5	5.56 (13.63)	-
35	NWG8	2.07	17.33 (24.47)	2.93	4.8	33.02 (35.07)	0.2	5.13	39.61 (35.07)	-	6.27	30.37 (33.44)	-
36	NWG9	2.17	13.33 (21.09)	2.83	4.5	37.21 (37.59)	0.5	5.5	35.29 (37.59)	-	6.1	32.22 (34.58)	-
37	NWG10	2.1	16.00 (23.58)	2.9	4.7	34.42 (35.92)	0.3	6.27	26.27 (35.92)	-	7.5	16.67 (24.09)	-
38	NWG11	1.8	28.00 (31.95)	3.2	5.2	27.44 (31.59)	-	5.2	38.82 (31.58)	-	5.5	67.04 (54.96)	-
39	NWG12	1.4	44.00 (41.55)	3.6	4.43	38.13 (38.13)	0.57	4.43	47.84 (38.13)	0.57	2.03	77.41 (61.62)	2.97
40	NWG13	1.8	28.00 (31.95)	3.2	5.23	26.98 (31.26)	-	5.23	38.43 (31.26)	-	6.37	29.26 (32.75)	-
41	NWG14	1.9	24.00 (29.33)	3.1	4.48	37.44 (37.72)	0.52	4.48	47.25 (37.72)	0.52	3.8	57.74 (49.45)	1.2
42	NWG15	2.2	12.00 (20.09)	2.8	4.8	33.06 (35.09)	0.2	5.2	38.82 (35.09)	-	5.33	40.74 (39.69)	-
43	NWG16	2.3	8.00 (13.51)	2.7	5.2	27.44 (31.59)	-	5.8	31.76 (31.59)	-	6.33	29.63 (32.98)	-
44	NWG17	1.8	28.00 (31.95)	3.2	5.1	28.83 (32.48)	-	6.07	28.63 (32.48)	-	7.1	21.11 (27.35)	-
45	NSK1	1.9	24.00 (29.28)	3.1	5.37	25.11 (30.07)	-	6.23	26.67 (30.07)	-	7.23	19.63 (26.30)	-
46	NSK2	1.93	22.67 (28.29)	3.07	5.2	27.44 (31.59)	-	7.43	12.55 (31.59)	-	9	0.00 (0.00)	-
47	NSK3	1.7	32.00 (34.45)	3.3	4.53	36.74 (37.31)	0.47	4.53	46.67 (37.31)	0.47	3.6	59.96 (50.75)	1.4
48	NSK4	1.8	28.00 (31.91)	3.2	4.8	33.02 (35.07)	0.2	5.23	38.43 (35.07)	-	5.33	40.74 (39.66)	-
49	NSK5	1.83	26.67 (31.08)	3.17	4.68	34.65 (36.06)	0.32	5.13	39.61 (36.06)	-	5.23	41.85 (40.31)	-
50	NSK6	2.17	13.33 (21.09)	2.83	5.03	29.76 (33.06)	-	5.63	33.73 (33.06)	-	5.67	37.04 (37.49)	-
	P.palmivora control	2.5	--	--	7.17	--	--	8.5	--	--	9	--	--
SEM ±		0.04	0.8		0.04	1.6		0.95	1.42		0.11	0.85	
CD (P≤ 0.05)		0.11	2.24		0.12	4.48		2.95	4.58		0.31	2.4	
CV (%)		3.6	5.81		2.36	11.6		4.25	8.99		3	4.89	

Figures in parenthesis are arc sine transformation values
Each treatment replicated thrice

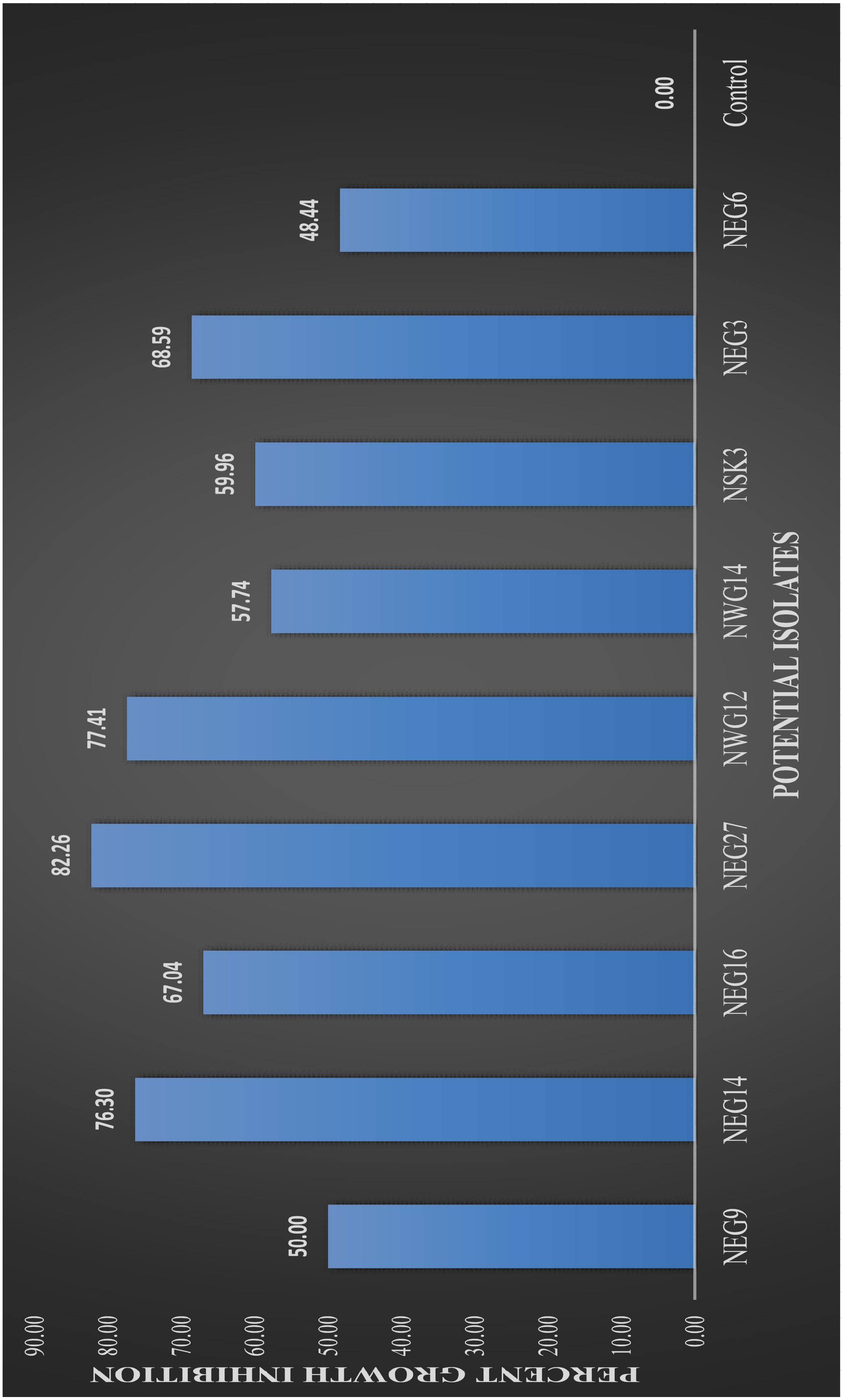

Fig. 4.8. Antagonistic efficacy of potential PGPR isolates against mycelial growth of *P. palmivora* under *in vitro*.

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Received on 25.12.2023 and Accepted on 05.02.2024