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Exploration of Indole Acetic Acid (IAA) Producing Fungi from Non-organic Carrot Rhizosphere as Biostimulant

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Abstract: This study aimed to isolate and characterize fungi from the rhizosphere of carrots grown in non-organic systems that potentially produce indole-3-acetic acid (IAA), and to evaluate their biostimulatory potential in supporting environmentally friendly agriculture. The methods employed included rhizospheric sample collection from non-organic carrot fields, fungal isolation and purification using PDA media, morphological identification, as well as qualitative screening and spectrophotometric quantification of IAA production using Salkowski reagent. The results revealed six fungal isolates capable of producing IAA, namely Rhizopus stolonifer, Rhizopus oryzae, Trichoderma longibrachiatum, Trichothecium roseum, Pythium aphanidermatum, and Pythium inflatum, with varying levels of IAA production. Among these, R. oryzae produced the highest IAA concentration at 46.8 ppm, followed by P. aphanidermatum (41.8 ppm), T. longibrachiatum (27.3 ppm), P. inflatum (24.5 ppm), R. stolonifer (18.4 ppm), and T. roseum (17.9 ppm). These findings suggest that rhizospheric fungi from the non-organic carrot rhizosphere hold promising potential as IAA-producing bioagents to support sustainable and environmentally friendly agricultural practices.

Keywords: Biostimulant; fungi; indole acetic acid; non-organic carrot; rhizosphere

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INTRODUCTION

Soil is a dynamic ecosystem inhabited by diverse microorganisms, many of which establish associations with plant roots (Iqbal et al., 2025). This environment provides ideal conditions that facilitate microbial interactions with plant root systems, playing essential roles in atmospheric regulation, nutrient cycling, enhancing plant productivity, disease suppression, and maintenance of ecosystem stability (Aqeel et al., 2023; Tharanath et al., 2024). A key zone involved in these interactions is the rhizosphere, a complex ecological niche surrounding plant roots that serves as a habitat for microorganisms supporting plant growth (Solomon et al., 2024). The structure and composition of rhizosphere microbial communities are shaped by root exudates, secretory products, and carbon dioxide released through root respiration (Chauhan et al., 2023). Additionally, environmental factors such as soil pH, texture, moisture, temperature, organic C, nutrient availability, soil type, and vegetation significantly influence microbial diversity (Brockett et al., 2012; Rousk et al., 2010; Zheng et al., 2019).

Cultivation practices also exert a substantial impact on soil microbial communities (Asghar et al., 2025; Omotayo & Babalola, 2021). Agricultural management systems, whether organic or non-organic, shape the dynamics of resident microorganisms (Bay et al., 2021). Organic farming, which emphasizes the use of cover crops, green manure, animal manure, and compost, tends to enhance microbial diversity in the soil

(Fess & Benedito, 2018). In contrast, non-organic systems that rely on synthetic fertilizers and pesticides often lead to a decline in beneficial microbial populations (B. Sharma et al., 2023). Nonetheless, certain microorganisms, including specific fungi and bacteria, exhibit tolerance to these chemical agents (Ayaz et al., 2023). *Trichoderma harzianum* and *Trichoderma viride* have been reported to tolerate fungicides such as Mancozeb, Thiram, and Copper oxychloride (Bagwan, 2010). *Aspergillus niger* can continue to grow despite exposure to Streptocycline (Chandra et al., 2016). Although microbial diversity in non-organic soils is generally lower, the persistence of Operational Taxonomic Units (OTUs) reflects microbial adaptability to such environments (Hartmann et al., 2015). Therefore, indigenous fungal with chemical tolerance may serve as promising biological agents in sustainable agricultural systems (Ramatsitsi et al., 2023), applicable to both organic and non-organic farming.

One horticultural crop that contributes to shaping rhizosphere microbial communities is the carrot (*Daucus carota* L.) (Anderson et al., 2024). This plant releases root exudates rich in carbohydrates, amino acids, and bioactive compounds, which attract soil microorganisms and promote rhizosphere colonization (Badri & Vivanco, 2009; Jambon et al., 2018). Fungal genera frequently found in the carrot rhizosphere include *Fusarium*, *Aspergillus*, *Trichoderma*, and *Penicillium* (Noviyanti et al., 2024; Srivastava et al., 2012). These fungi are recognized as Plant Growth-Promoting Fungi (PGPF) for their ability to protect plants from pathogens, enhance nutrient availability, and stimulate physiological functions through biostimulant activity (Adedayo & Babalola, 2023; Hossain & Sultana, 2020). As biostimulants, PGPF facilitate plant growth by releasing compounds that influence physiological processes, including the synthesis of plant growth hormones (El-Maraghy et al., 2021; M. Sharma et al., 2024).

Among the most prominent plant growth hormones is auxin, particularly Indole Acetic Acid (IAA) (Etesami & Glick, 2024). IAA is a crucial phytohormone essential for supporting plant growth, especially in shoot development, as it promotes cell division and differentiation. Moreover, IAA plays a pivotal role in cell elongation, lateral root formation, and the regulation of gravitropism and phototropism (Roopa et al., 2023). As the main naturally occurring auxin, IAA offers a more environmentally sustainable approach to promoting plant growth compared to synthetic hormone analogs. Fungal genera such as *Trichoderma* and *Penicillium* have been widely reported to produce significant quantities of IAA. Larekeng et al. (2019), revealed that fungal isolates from the mahogany rhizosphere were capable of producing IAA, with *Trichoderma* exhibiting the highest concentrations. Likewise, Imaningsih et al. (2021) isolated IAA-producing fungi from the rhizosphere of *Melaleuca* trees in peatland areas, including the genera *Penicillium* and *Syncephalastrum*. These findings highlight the potential of natural fungal isolates as PGPF and biostimulant agents.

Despite the increasing research in the field, studies on the potential of rhizosphere fungi from non-organic carrot fields as IAA producers remain scarce. This lack of data impedes efforts to optimize the role of PGPF in non-organic agricultural systems. Investigating this potential can drive innovations in sustainable agriculture, especially in farms dependent on synthetic inputs. This study aimed to isolate and characterize fungi from the rhizosphere of carrots grown in non-organic systems that potentially produce IAA and to evaluate their biostimulatory potential in supporting environmentally friendly agriculture.

METHOD

Study Site and Duration

This research was conducted at the Biotechnology Laboratory, Department of Biology, Faculty of Science and Mathematics, Diponegoro University, from July to October 2023. Soil samples were collected from a conventionally cultivated carrot field located in Kejajar Village, Wonosobo Regency, Central Java.

Equipment and Materials

The equipment used in this study included an analytical digital balance, shovel, measuring glass, vortex mixer, erlenmeyer flasks, microwave, oven, autoclave, graduated pipettes, test tubes, test tube racks, bunsen burner, petri dishes, inoculating needle, camera, centrifuge, glass slides, cover slips, microscope, cuvettes, spectrophotometer, fungal identification guidebook, and stationery. The materials used consisted of carrot rhizosphere soil, potatoes, glucose, agar, chloramphenicol, 70% alcohol, 10% Clorox/NaOCl, synthetic Indole Acetic Acid (IAA), salkowski reagent, L-tryptophan, plastic wrap, labels, cotton, plastic bags, and sterile distilled water.

Research Procedure

Sample collection

Soil sampling was conducted by collecting soil adhering to the roots of healthy carrot plants at a depth of approximately 10–15 cm from the soil surface. The rhizosphere soil was then placed into clean plastic bags and labeled with information regarding the plant source, sampling location, and collection date.

Preparation of growth media

This study utilized two types of enriched fungal growth media: Potato Dextrose Agar (PDA) and Potato Dextrose Broth (PDB). To prepare PDA, 200 grams of potatoes were peeled and finely chopped, then boiled in 1 liter of sterile distilled water until fully cooked. The boiled mixture was filtered, and the final volume was brought back to 1 liter using distilled water. Afterward, 20 grams of glucose, 0.05 grams of chloramphenicol, and 15 grams of agar were added to the filtrate. The mixture was homogenized and sterilized in an autoclave at 121°C for 15 minutes. Meanwhile, PDB was prepared following the same procedure as PDA but without the addition of agar. After homogenization, the broth was also sterilized in an autoclave at 121°C for 15 minutes.

Isolation and purification of rhizospheric fungi

Fungal isolation from rhizospheric soil was performed using a serial dilution method. The process began by weighing 1 gram of the soil sample on an analytical digital balance and transferring it into a test tube containing 9 mL of sterile distilled water. The mixture was homogenized using a vortex mixer for several minutes to create a 10^{-1} dilution suspension. Afterward, 1 mL of the suspension was transferred into another test tube containing 9 mL of sterile distilled water and homogenized again to produce a 10^{-2} dilution. This process was repeated until a 10^{-3} dilution was achieved. A 0.5 mL aliquot from each suspension was pipetted onto a sterile PDA agar plate and gently swirled to ensure even distribution. The Petri dishes were labeled and incubated at room temperature for approximately 4 days. Purification was carried out by transferring a single fungal colony from the isolation plates to fresh PDA medium using a sterile inoculating needle. Incubation continued at room temperature for approximately 3 days to obtain pure isolates.

Preliminary screening for IAA-producing fungi

The purified fungal isolates were tested for their ability to produce the hormone Indole Acetic Acid (IAA) using sterile Potato Dextrose Broth (PDB) enriched with L-

tryptophan. The medium was distributed into 50 mL Erlenmeyer flasks and inoculated with a loopful of fungal conidia. The flasks were placed on a shaker and incubated at 100 rpm for 5 days at room temperature until discernible fungal growth was evident. After incubation, 5 mL of the culture medium containing the fungal isolate was transferred into a centrifuge tube and centrifuged at 8000 rpm for 30 minutes. From the resulting supernatant, 4 mL was collected and mixed with 1 mL of Salkowski reagent. The mixture was incubated in the dark for 30 minutes.

Quantification of IAA production

The concentration of IAA hormone was determined using a spectrophotometer at a wavelength of 520 nm. Synthetic IAA solutions at concentrations of 50, 60, 70, 80, 90, and 100 ppm were used as references to generate a standard curve, and their absorbance was measured. The resulting absorbance data were then used to plot the standard curve and derive the linear regression equation ($y = ax + b$) using Microsoft Excel software. The absorbance value of the supernatant from the fungal isolate was subsequently measured and inserted into the regression equation to calculate the corresponding IAA concentration (x). This method enabled the quantitative assessment of IAA hormone levels produced by each isolate.

Morphological identification of fungal isolates

Fungal isolates were identified based on macroscopic and microscopic characteristics after the isolates had been grown on PDA medium for 1–2 weeks. The identification process was conducted after purification of the isolates by comparing their characteristics with fungal identification guidebook from Compendium of Soil (Domsch et al., 1980), The Pictorial Atlas of Soil and Seed Fungi: Morphologies of Cultured Fungi and Key to Species (Watanabe, 2010), Food and Indoor Fungi (Samson et al., 2010), and other supporting literature. Macroscopic characteristics observed included colony diameter, color, and surface texture. Microscopic observations focused on the morphology of hyphae, spores, and other distinctive structures specific to each fungal species. Microscopic examinations and structural measurements were performed using Optilab version 2.2 and Image Raster 3 software. The identification results were interpreted to determine the fungal species based on the observed traits.

RESULTS AND DISCUSSION

Isolation and Preliminary Screening of Rhizospheric Fungi

A total of 15 fungal isolates were obtained from the non-organic carrot rhizosphere. These isolates consisted of 10 colonies from the 10^{-1} dilution, 4 colonies from the 10^{-2} dilution, and 1 colony from the 10^{-3} dilution. Each colony exhibited distinct macroscopic characteristics and was assigned the code “JRWN”.

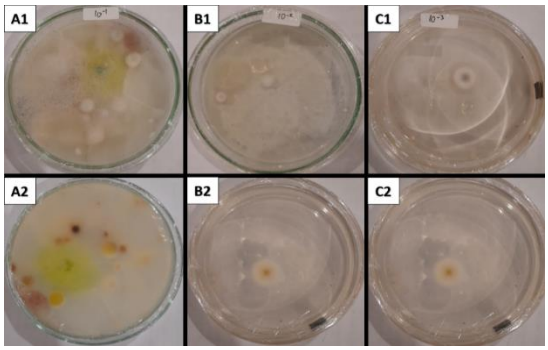

Figure 1. Rhizospheric fungal colonies from serial dilutions: 10^{-1} (A1: top, A2: reverse), 10^{-2} (B1: top, B2: reverse), and 10^{-3} (C1: top, C2: reverse)

Among the 15 isolates tested, 6 demonstrated the ability to produce the hormone Indole Acetic Acid (IAA). This was indicated by a color change from yellow (control) to orange in the medium containing Salkowski reagent, as shown in Figure 2.

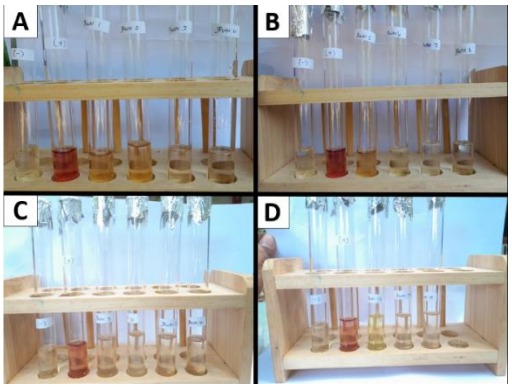

Figure 2. Screening results, control (-) and control (+), from left: A: JRWN 1-4. B: JRWN 5-8. C: JRWN 9-12. D: JRWN 13-15

The observed orange color change in the medium inoculated with fungal isolates was caused by a reaction between IAA and the Fe components in the Salkowski reagent. Fe^{3+} in the reagent interacts with the indole group in IAA, forming a red complex compound $[Fe_2(OH)_2(IA)_4]$. This oxidative reaction leads to a color change that intensifies with increasing IAA concentration. A more intense red color provides a qualitative indication of higher IAA production by the fungal isolates. Based on these results, the 6 isolates found to produce IAA are: JRWN 1, JRWN 2, JRWN 3, JRWN 4, JRWN 5, and JRWN 9.

Morphological Characteristics of Fungal Isolates

The 6 fungal isolates from the non-organic carrot rhizosphere exhibited diverse morphological features, as observed from their macroscopic and microscopic characteristics, as presented in Table 1.

Table 1. Macroscopic and microscopic characteristics of fungi

Description	JRWN					
	1	2	3	4	5	9
Macroscopic						
Color (Upper)	Brown	Brown-greyish	Olive green	Grey-pinkish	White	White
Color (Reverse)	Greyish	Greyish	Pale yellow	Yellow	White	White
Growth rate*	Rapid	Rapid	Rapid	Moderate	Rapid	Rapid
Texture	Cottony	Cottony	Granular	Velvety	Cottony	Cottony
Pigment releasing	-	-	Yellow	-	-	-
Microscopic						
Hyphae	Hyphae	Aseptate	Aseptate	Septate	Septate	Aseptate
Rhizoid color	Brown	Brown	-	-	-	-
Sporangiophore color	Brown	Brown	-	-	-	-
Sporangiophore wall	Smooth	Smooth	-	-	-	-
Sporangium shape	Globose	Globose	-	-	Lobate	Lobate
Sporangium color	Dark brown	Dark brown	-	-	Hyaline	Hyaline
Sporangium texture	Smooth	Smooth	-	-	Smooth	Smooth
Columella shape	Globose	Subglobose	-	-	-	-
Columella color	Brown	Brown	-	-	-	-
Sporangiospore shape	Ovoid, subglobose	Ovoid	-	-	-	-
Sporangiospore texture	Striate	Striate	-	-	-	-
Chlamydospore	-	Present	Present	-	-	-
Chlamydospore shape	-	Globose	Subglobose	-	-	-

Description	JRWN					
	1	2	3	4	5	9
Conidiophore	-	-	Hyaline	Hyaline	-	-
Conidiophore branching	-	-	Branching	Not branching	-	-
Conidia shape	-	-	Ellipsoidal, ovate	Ellipsoidal, pyriform	-	-
Conidia color	-	-	Greenish	Hyaline	-	-
Conidia cell	-	-	Single	Two	-	-
Conidia texture	-	-	Smooth	Smooth	-	-
Oogonia location	-	-	-	-	Terminal	Intercalary
Amount of Antheridium	-	-	-	-	1-2	2-3

Note: (*) rapid growth rate: diameter 7-9 cm^2 in 4 days, moderate growth rate: diameter 4-6 cm^2 in 4 days, slow growth rate 1-3 cm^2 in 4 days.

Each isolate exhibited distinct colony colors, surface textures, and growth rates on Potato Dextrose Agar (PDA), highlighting variation at the macroscopic level. At the microscopic level, differences were noted in hyphal types, sporangial structures, as well as the shape and texture of spores. This morphological diversity suggests that the isolates likely belong to different genera and reflects their adaptive responses to the environmental conditions of the rhizosphere. These observations form a crucial foundation for the species identification and further taxonomic of the isolates.

Identification of Fungal Isolates

1. Rhizopus stolonifer (JRWN 1)

Macroscopic observations on PDA medium after 7 days of incubation showed that the colonies grew rapidly and completely covered the surface of the medium by day 4. The colony color transitioned from white to brown, with the reverse side appearing greyish and exhibited a cotton-like texture. Microscopically, the sporangiophores were solitary or arranged in groups of 3–8, measured 250–2800 µm in length and 10–30 µm in diameter, and appeared brown, straight, aseptate, with smooth walls. Rhizoids were repeatedly branched, ranging from hyaline to brown, and well developed. The sporangia were globose, measured 78–230 µm in diameter, while the columellae were globose to subglobose, with diameters ranging from 53–119 µm. Sporangiospores were ovoid to subglobose, measured 4–10 µm in diameter, and had striate surface ornamentation. No chlamydospores were observed at 37°C (Figure 3). These morphological features are consistent with the descriptions of *Rhizopus stolonifer* reported by Domsch et al. (1980) and Samson et al. (2010), as corroborated by the observations of Sari et al. (2024) and Liu et al. (2024).

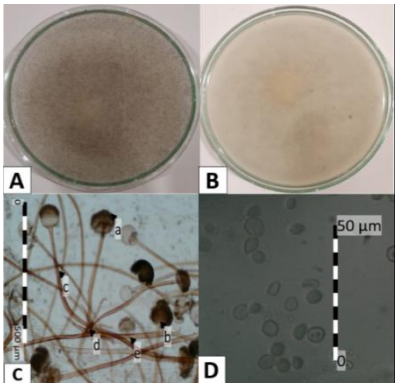

Figure 3. Morphological characteristics of *Rhizopus stolonifer*. A: Upper Colony, B: Reverse Colony, C: (a. Sporangium, b. Columella, c. Sporangiophore, d. Rhizoid, e. Stolon), D: Sporangiospore

2. Rhizopus oryzae (JRWN 2)

Macroscopic observations on PDA medium after 7 days of incubation revealed that the colonies grew rapidly. By day 4, they had completely covered the surface of the medium, and the colony color changed from white to brown-greyish. The reverse side of the colony appeared gray and exhibited a characteristic cottony texture. Microscopically, sporangiophores were brown, aseptate, straight, and occurred either solitarily or in groups of 2–5, measured 280–1550 µm in length and 6–20 µm in diameter, with smooth surfaces. Some sporangiophores showed localized swellings. The rhizoids were singly branched or finger-like, brown, and relatively short. The sporangia were brown to dark brown, globose to subglobose in shape, with diameters ranging from 58 to 184 µm. The columellae were brown, subglobose, and measured 39–135 µm in diameter. The sporangiospores were ovoid, 4–9 µm in diameter, and exhibited striate surface ornamentation. This isolate also produced numerous chlamydospores at 37°C, which were thick-walled, hyaline, and irregular to globose or ovoid in shape, with diameters of 10–30 µm (Figure 4). These morphological characteristics are consistent with the descriptions of *Rhizopus oryzae* reported by Domsch et al. (1980) and Samson et al. (2010),, as corroborated by the observations of Anchundia et al., 2024) and (Ardiani et al., 2024).

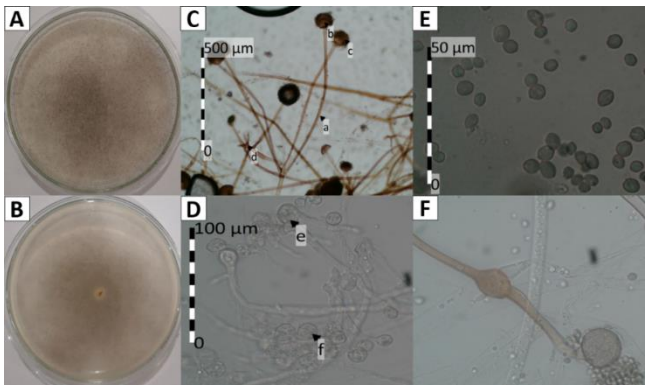

Figure 4. Morphological characteristics of *Rhizopus oryzae*. A: Upper colony, B: Reverse colony, C: (a. Sporangiophores, b. Columella, c. Sporangium, d. Rhizoids), D: (e. Hyphae Swelling, f. Chlamydospores), E: Sporangiospores, F: Sporangiophore swelling

3. Trichoderma longibrachiatum (JRWN 3)

Macroscopic observations on PDA medium over 7 days incubation period revealed that the colonies spread rapidly. By day 5, the colonies had completely covered the surface of the medium, with a color transition from white to greenish, accompanied by the initial release of a yellow pigment. This color change was resulted from conidial production. By day 7, the colonies appeared olive green, with increasingly intense yellow pigmentation diffusing into the growth medium. The reverse side of the colony was pale yellow, and the texture was granular. Microscopic examination showed that the isolate possessed septate hyphae with few but elongated branches. Primary branches were longer than the secondary ones, ranging from 250 to 1400 µm in length. Phialides were solitary, flask-shaped, straight, and measured 8–70 µm in length, each arising directly from a branch. Conidia were produced at the tips of the phialides (phialosporous), ovate to ellipsoidal in shape, smooth-walled, and measured 2.5–8 µm in diameter. This isolate also produced numerous chlamydospores after more than two weeks, which were subglobose, thick-walled, and measured 6–11 µm in diameter (Figure 5). These morphological features are consistent with the

descriptions of *Trichoderma longibrachiatum* provided by Rifai, (1969) and Bissett, (1984), and are further supported by the observations of Kumar et al. (2019).

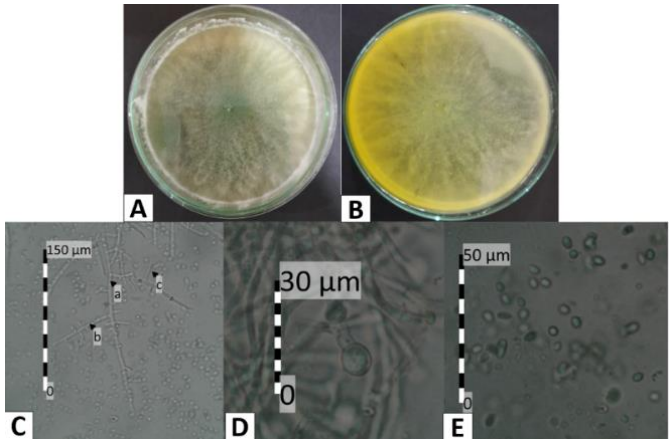

Figure 5. Morphological characteristics of *Trichoderma longibrachiatum*. A: Upper colony, B: Reverse colony, C: (a. Main Branch, b. Secondary Branch, c. Phialid), D; Chlamydospores, E: Conidia

4. Trichothecium roseum (JRWN 4)

Macroscopic observations on PDA medium over a 7-day incubation period showed that the colony exhibited slow growth. By day 3, the colony had reached a diameter of 3.4 cm, which expanded to 6.2 cm by day 7. The colony color began shifting from white to gray on day 3, and by day 6, a faint pink hue became visible. The reverse side of the colony was initially white on day 2 but turned yellow by day 4. The colony exhibited a velvety texture. Microscopic examination revealed that the isolate possessed septate hyphae and erect, unbranched conidiophores ranging 30–300 µm in length and 3–5 µm in width. Conidia were produced at the tips of the conidiophores, typically in groups of 2–8. They were ellipsoidal to pyriform in shape, hyaline, with a broader upper part and a narrower base. Each conidium was two-celled, thick-walled, and measured 9–20 µm in size (Figure 6). These morphological characteristics are consistent with the descriptions of *Trichothecium roseum* provided by Domsch et al. (1980), Barnett & Hunter, (1987), Watanabe (2010), and are further supported by the observations of Oh et al. (2014).

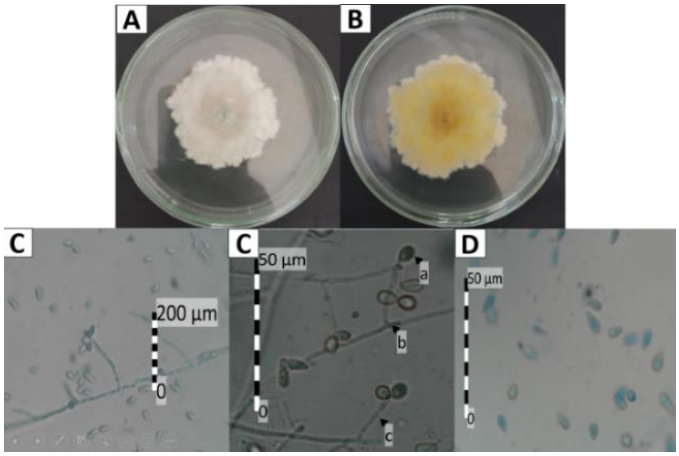

Figure 6. Morphological characteristics of *Trichothecium roseum*. A: Upper colony, B: Reverse colony, C: Conidiophores with Conidia at the End, C: (a. Conidia, b. Foot cell, c. Conidiophores), D: Conidia

5. Pythium aphanidermatum (JRWN 5)

Macroscopic observations on PDA medium over 7 days incubation period showed rapid colony growth. By day 4, the colony had covered the entire surface of the medium. The colony appeared white, with a white reverse side, exhibiting a cottony texture and forming a radiate pattern. Microscopic examination revealed that the isolate contained non-septate hyphae. The sporangia were lobate and branched, measured 30 to 45 µm. Oogonia were terminal, with thick walls and diameters ranging from 19 to 25 µm. Antheridia were one to two per oogonium, measuring 10 to 20 µm in length (Figure 7). These morphological characteristics align with the descriptions of *Pythium aphanidermatum* provided by Matthews (1932), Domsch et al. (1980), Plaats-Niterink (1981), and Watanabe, (2010), as corroborated by the observations of Ashwathi et al. (2017).

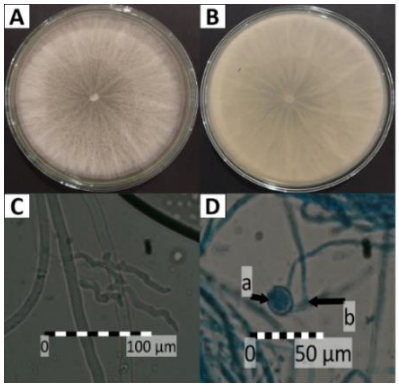

Figure 7. Morphological characteristics of *Pythium aphanidermatum*. A: Upper colony, B; Reverse colony, C: Lobate Sporangium, D: (a. Oogonia, b. Antheridia)

6. Pythium inflatum (JRWN 9)

Macroscopic observations on PDA medium over a 7-day incubation period showed rapid colony growth. By day 4, the colony had covered the entire surface of the growth medium in the Petri dish. The colony appeared white, with a white reverse side, and exhibited a cottony texture. Microscopic examination (Figure 8) revealed that this isolate possessed non-septate hyphae. The sporangia were lobate with irregular shapes, containing abundant protoplasm, and measured 23–68 µm in diameter. Oogonia were terminal or intercalary, with thick walls and diameters ranging from 19 to 24 µm. Antheridia were one to three per oogonium, and 7–11 µm in length (Figure 8). These morphological characteristics align with the descriptions of *Pythium inflatum* provided by Matthews (1932), Plaats-Niterink (1981), and Watanabe, (2010), and are further supported by the observations of (Nam & Choi, 2019).

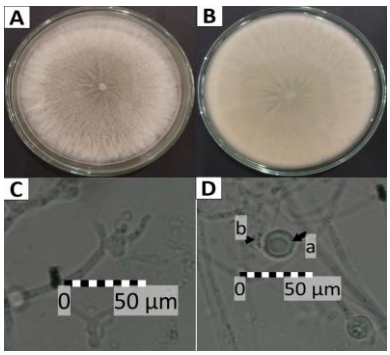

Figure 8. Morphological characteristics of *Pythium inflatum*. A: Upper colony, B: Reverse colony, C: Sporangium, D: (a. Oogonia, b. Antheridia)

IAA Production Potential of Fungal Isolates

Absorbance was measured using a spectrophotometer at a wavelength of 520 nm, and the results are presented in Table 2. Based on the standard IAA curve (Figure 9), the linear equation y = 0.0018x - 0.0002 was obtained with an R^2 value of 0.9993, indicating a very strong correlation between absorbance and IAA concentration.

Table 2. Concentration and absorbance of synthetic IAA for standard curve

Concentration (ppm)	Absorbance
50	0,088
60	0,107
70	0,124
80	0,142
90	0,158
100	0,178

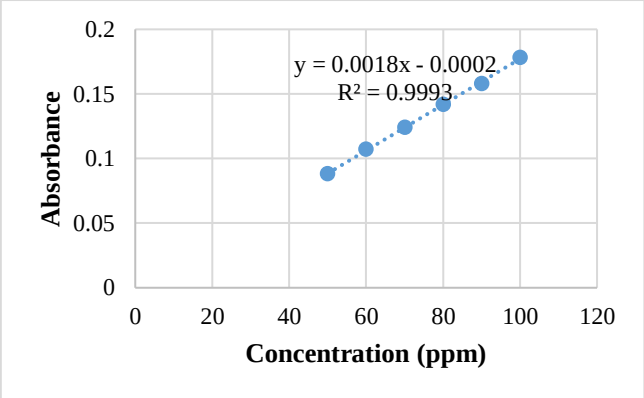

Figure 9. Linear curve graph between concentration and absorbance of synthetic IAA for standard curve

The IAA concentration produced by each fungal isolate was calculated by substituting the absorbance values into the standard curve equation. The results are shown in Table 3.

Table 3. Concentration measurements of rhizosphere fungal isolates

Isolate	Absorbance	IAA Concentration (ppm)
Rhizopus stolonifer	0,033	18,4
Rhizopus oryzae	0,084	46,8
Trichoderma longibrachiatum	0,049	27,3
Trichothecium roseum	0,032	17,9
Pythium aphanidermatum	0,075	41,8
Pythium inflatum	0,044	24,5

The measurements revealed that each fungal isolate displayed a different ability to produce IAA. *Rhizopus oryzae* produced the highest concentration, 46.8 ppm, followed by *Pythium aphanidermatum* (41.8 ppm), *Trichoderma longibrachiatum* (27.3 ppm), *Pythium inflatum* (24.5 ppm), *Rhizopus stolonifer* (18.4 ppm), and *Trichothecium roseum* (17.9 ppm).

The highest IAA concentration produced by *Rhizopus oryzae* (46.8 ppm) was higher than those reported in several previous studies. (Larekeng et al., 2019) reported IAA production by *Penicillium* at 19.96 ppm and *Trichoderma* at 20.36 ppm from mahogany rhizosphere. Additionally, Gusmiaty et al. (2019) observed IAA production of 38.6 ppm in the *Fusarium* genus from suren tree rhizosphere, while (Imaningsih et al., 2021) found that *Penicillium* sp. from the galam rhizosphere produced only 8.46 ppm.

The variation in IAA production among the isolates, even within the same genus, reflects differences in their metabolic abilities to synthesize IAA from L-tryptophan as a precursor. IAA concentration is also influenced by the amount of L-tryptophan in the medium. The addition of tryptophan has been shown to increase IAA biosynthesis by up to 2.7 times, due to enhanced enzymatic activity in the IAA synthesis pathway (Suebrasri et al., 2020). IAA synthesis using L-tryptophan as a precursor can occur through three metabolic pathways: 1) Oxidation of L-tryptophan by tryptophan-2-monooxygenase, forming indole-3-acetamide, which is subsequently hydrolyzed by indole-acetamide hydrolase into indole-3-acetic acid (IAA); 2) Decarboxylation of L-tryptophan into tryptamine by tryptophan decarboxylase, followed by oxidation of tryptamine by amine oxidase into indole-3-acetaldehyde, which is then converted into indole-3-acetic acid (IAA) by indole-acetaldehyde dehydrogenase; 3) Transamination of L-tryptophan into indole-3-pyruvic acid by tryptophan transaminase, followed by decarboxylation by indole-3-pyruvate decarboxylase into indole-3-acetaldehyde, and final conversion into indole-3-acetic acid (IAA) by indole-acetaldehyde dehydrogenase (Lehmann et al., 2010; Li et al., 2018; Patten et al., 2013). L-tryptophan is also an exudate from plant roots that influences the microbial community structure in the rhizosphere. Microorganisms, including fungi, utilize this exudate for IAA synthesis, which is then absorbed back by the plant roots and plays a role in stimulating cell division and shoot growth (Swamy et al., 2016).

This study demonstrates that rhizosphere fungi from non-organic carrot soil have high potential for IAA hormone production. This can be attributed to the nature of carrots as tuberous plants that store photosynthetic products in their roots and produce high levels of L-tryptophan, thus attracting IAA-producing microorganisms (Noor et al., 2023). These findings suggest that the rhizosphere of non-organic carrots is a potential source of IAA-producing microbes, which could be developed as biological agents to support sustainable and environmentally friendly agricultural systems in the future.

CONCLUSION

Based on the research results, it can be concluded that (1) Rhizosphere fungi isolated from non-organic carrot fields exhibited varying capacities to produce indole-3-acetic acid (IAA); (2) *Rhizopus oryzae* produced the highest concentration of IAA at 46.8 ppm, followed by *Pythium aphanidermatum* (41.8 ppm), *Trichoderma longibrachiatum* (27.3 ppm), *Pythium inflatum* (24.5 ppm), *Rhizopus stolonifer* (18.4 ppm), and *Trichothecium roseum* (17.9 ppm); (3) These findings suggest that fungal isolates from the non-organic carrot rhizosphere hold promising potential as IAA-producing bioagents to support natural and sustainable plant growth.

RECOMENDATION

Further studies are recommended to explore the IAA biosynthesis mechanisms in each fungal isolate, particularly *Rhizopus oryzae* and *Pythium aphanidermatum*, which exhibited the highest production levels, by identifying the genes or enzymes involved in the biosynthetic pathways and investigating the effects of varying L-

tryptophan concentrations on hormone production, in order to support sustainable agriculture.

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