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PLANT GROWTH PROMOTING AND DISEASE PREVENTION PROPERTIES OF ENDOPHYTIC AND RHIZOPLANE BACTERIA ISOLATED FROM *HEMIDESMUS INDICUS*- A RARE ENDANGERED MEDICINAL PLANT

Research article

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ABSTRACT

Endophytic and rhizoplane are beneficial bacteria that colonize plant and enhance the growth and disease resistance ability through various mechanisms. The application of growth promoting endophyte and rhizoplane is steadily increasing as an alternative to chemical fertilizers and pesticides. These organisms have greater role to play in the ecological adaptation of plants which are considered to be on the verge of becoming rare and endangered. Also microorganisms from medicinal plant are of great importance as these organisms are reported to produce metabolites which are of medicinal value. The objective of this study is to isolate the endophytic and rhizoplane bacterial strain which have growth promoting characteristic as well as phytopathogen suppressing ability from *Hemidesmus indicus,* a medicinal plant which is considered as rare and endangered as a reason of overexploitation because of its medicinal value. In the current study we have been able to isolate 11 endophytic and 6 rhizoplane bacteria. Among them 8 endophytes and 3 rhizoplane isolates are showing significant antifungal activity. This suggests the role these organisms play in disease resistance and can be used as potential bio control agents.

Introduction:

Endophytic and Rhizoplane bacteria are the organisms which dwell inside and on the surface of plant body without causing any harmful effect to plants. They exhibit strong interaction and have symbiotic association with their host **(Misaghi et al, 1990)**. These microorganisms have i) Growth promoting abilities ii) enhance disease resistance against phytopathogens and iii) increase mineral absorption capacity of plants. **(Sturz *et al.*, 1999; Nejad *et al.*, 2000)**. These beneficial effects of endophytic and rhizoplane bacteria occur through various mechanisms including; i) nitrogen fixation ii) solubilizaton of phosphate ii) siderophore production iv) synthesis of growth hormones like indole acetic acid and cytokinin v) hydrogen cyanide production and vi) release of antimicrobial compounds **(Ngoma *et al.*, 2013)**.

Nitrogen fixation is achieved due to the presence of nifH gene in paenibacillus polymyxa (Pp) bacteria. In addition to this, bacteria is capable of producing growth hormone cytokinin. The growth promotion ability of cytokinin hormone released by Pp bacteria has been demonstrated in Arabdinosis thaliana **(Ryu *et al.*, 2005; Timmusk *et al.*, 1999)**. Phosphorous is one of the important plant nutrient required for physiological activities like cell division, photosynthesis and development of root system. Major portion of phosphate present in soil is immobilized because of insoluble organic and inorganic matter. Endophytic and rhizoplane bacteria posses the ability to solubilize the

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immobilized mineral phosphate by releasing organic acids and phosphate enzyme **(Paul *et al.*, 2013; Sharma et al., 2011).** Siderophore, an iron chelating compound which is released from fluorescent pseudomonas, extracts iron from rhizoshere and makes it available to plants. Iron is one of the important element required by plants for various biochemical activities such as electron transport system, formation of heme, as a cofactor for enzymes and synthesis of chlorophyll **(Deshwal *et al.*, 2013).** Indirect way through which endophytes can promote growth is by protecting the plants from pathogens. These endophytic and rhizoplane bacteria produce certain antifungal compounds which gives these plants disease resistance ability. For instance introduction of growth promoting bacteria into rhizosphere enhanced the growth rate of capsicum annum and also increased its disease resistance ability **(Kokalis *et al.*, 2006)**.

Hemidesmus (H). indicus is a slender, laticiferous and twining shrub, sometimes prostrate other times semi-erect with slender stem thickened at the nodes. The leaves are opposite, short-petiole, very variable, elliptic-oblong to linear lanceolate. It is widely recognized in folk medicine and as ingredient in Ayurvedic and Unani preparations against diseases of biliousness, blood diseases, diarrhoea, skin diseases, respiratory diseases, fever, bronchitis, eye diseases, burning sensation, rheumatism and gastric disorders **(Austin, 2008; Ravishankara *et al.*, 2004 and Mary *et al.*, 1987)**. Extract of this plant is reported to possess anti-inflammatory, antipyretic, antioxidant and antiulcerogenic properties **(Mary *et al.*, 1987; Alam, Gomes, 1998; Anoop, Jegadeesan, 2003)**. *H. indicus* extract is also found to inhibit lipid peroxidation and scavenge hydroxide radicals **(Amirghofran, 2000).** Endophytes from medicinal plants have become a hot topic for metabolite discovery because of their high biodiversity, predicted potential to produce novel compounds, involvement in growth promotion and enhancement of disease resistance ability in plants. **(Ryu *et al.*, 2005)**. There exist many medicinal plants being unexplored for the endophyte and rhizoplane communities. *H. indicus* is one among them. The aim of current study is to isolate and examine the plant growth promoting properties and disease prevention properties of endophytic and rhizoplane bacteria from the medicinal plant *H. indicus*.

Methods:

H. indicus plant samples were collected from Art of Living International Centre, Bengaluru. Roots were cut in to 1 cm long section and sterilized as follows: washed initially with running tap water for 10 minutes to remove soil, dirt & debris adhering to it then it was treated with 5% sodium hypochlorite solution for 10min; with 70% ethanol for 2 min; washed in sterile double distilled water for three times. The efficacy of sterilization was confirmed by spreading 0.1 mL of final washed solution on the surface of Tryptone soy agar medium (TSA). There was no growth witnessed after 24 hours of incubation indicating the sterility of final solution. Root samples were cut into smaller pieces and inoculated on TSA, Nutrient agar media, Kings B media, Soil extract media and Rovira medium. These plates were incubated at 28℃ for 48-72 hours. Pure cultures were obtained with streaking method. **(Misaghi, 1990; Zinniel, *et al.*, 2002; Anjum *et al.*, 2015)**. 10cm long root surface was rinsed in sterile petridish containing 10 mL of sterile saline phosphate buffer (pH 7.4) by gently rubbing with sterile painting brush to harvest adhered bacterial community before surface sterilization. The washed saline phosphate buffer was considered as source of rhizoplane bacteria and 0.1 mL of source was inoculated on TSA, Nutrient agar media, Kings B media, Soil extract media and Rovira media. **(Kokalis *et al.*, 2006).** Some of the biochemical tests conducted to determine the growth promotion and antifungal property of endophytic and rhizoplane bacteria are as follows.

Siderophore production test: (Gamit *et al.*, 2014)

Succinic acid is the media used for siderophore production test. Composition of Succinic acid media are as follows: K_2HP0_4, 6.0; KH_2P0_4, 3.0; $(NH4)_2S0_4$, 1.0; $MgS0_4.7H_20$, 0.2; succinic acid, 4.0. Above chemicals were dissolved in 1000ml of distilled water. The pH was adjusted to 7.0 by addition of 1N NaOH prior to sterilization. Enriched endophytic and rhizoplane cultures were inoculated into above media and incubated at 37° C with constant shaking for 24 to 48 hours. Appearance of green color indicates the presence of siderophore.

Phosphate solubilization test: (Goldstein, 1986)

The test is conducted by growing the cultures on the solid media which has phosphate as one of the ingredient. Bacterial plates were kept for incubation at 30° C for 2–3 days. The colonies forming halo zone of clearance around them were counted as Phosphate

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solubilizers. Media include following components: Glucose–10, NH_4Cl–1, $MgSO_4.7H_2O$–1, Agar–20. 50 mL of 10% K_2HPO_4 and 100 mL of 10% $CaCl_2$ were autoclaved separately and added later.

Indole Acetic acid (IAA) test: Tryptone media: (Deshwal *et al.*, 2013)

Qualitative indole production was determined using kovav's reagent (HCL + Dimethylaminobenzaldehyde in amyl alcohol). Formation of cherry red color indicates the presence of indole. Loop full of inoculum was added to tryptophan media and incubated for 24 hours and culture was used for the test.

Vogues proskeur test: (Hemraj *et al.*, 2013)

Bacterial isolates were inoculated into MR–VP media and incubated at 35° C for 24–48 hours. 1ml of the culture was aseptically transferred into clean, sterile test tubes. 15 drops of VP reagent A was added followed by 5 drops of VP reagent B. Red color within 20 minutes indicates the positive result.

Methyl red Test: (Hemraj *et al.*, 2013)

24–48 hours old bacterial cultures were taken in clean, sterile test tubes and added with 5 drops of Methyl red indicator. Color change to bright red indicates the positive result.

Hydrogen cyanide (HCN) production test: (Ngoma *et al.*, 2013)

Bacterial isolates were grown in 10% Tryptone Soya Agar media containing 4.4g/l of glycine. A Whatman filter paper No.1 was soaked in 2% sodium carbonate and 0.5% picric acid solution was placed on the underside of petridish lids. Plates were sealed with parafilm to avoid escape of gases. Plates were kept for incubation at room temperature for 5 days. Color change of filter paper from Yellow to reddish brown indicates the production of HCN.

Antifungal activity (Dual Culture Method): (Patel *et al.*, 2012)

Fungal pathogen Phytophthera grown on Potato Dextrose Agar (PDA) media was taken and inoculated at the centre on PDA plates containing 10% TSA. Bacterial culture was streaked on both sides of the fungal pathogen. This was sealed and incubated at room temperature for 4–5 days and examined for zone of inhibition.

Catalase test: (Guptha *et al.*, 2015)

24 hour old bacterial culture was taken on a glass slide and a drop of H_2O_2 was added. Rapid evolution of oxygen as evidenced by bubbling within 5-10 sec is considered as positive catalase test.

Results:

Total number of endophytic bacteria isolated were 11 which are named as follows ENBHI1, ENBHI2, ENBHI3, ENBHI4, ENBHI5, ENBHI6, ENBHI7, ENBHI8, ENBHI9, ENBHI10 and ENBHI11 and the number of rhizoplane bacteria isolated were 6 which are named as RPBHI1, RPBHI2, RPBHI3, RPBHI4, RPBHI5 and RPBHI6. List of Biochemical tests conducted and their results are shown in **Tables 1 and 2.**

Tables 1: Biochemical test results for Endophytic bacteria isolated from *H. indicus*

Endophytic Bacterial strain	Indole test	Siderophore production test	Phosphate solubilization test	Methyl red test	Voges-proskauer test	Catalase test	Dual culture	HCN production test
ENBHI1	-	-	-	-	-	-	+	-
ENBHI2	+	-	-	-	-	-	-	-
ENBHI3	-	+	-	-	-	+	+	+
ENBHI4	-	-	+	+	+	+	+	+
ENBHI5	-	-	+	-	+	+	+	-
ENBHI 6	-	-	-	+	-	-	-	-
ENBHI7	-	-	-	-	+	-	+	-
ENBHI8	-	-	-	-	-	+	+	-
ENBHI9	-	+	-	-	-	-	+	-

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ENBHI10	-	-	+	+	+	+	+	+
ENBHI11	-	-	-	-	-	+	-	-

Table 2: Biochemical test results for Rhizoplane bacteria isolated from *H. indicus*

Rhizoplane bacterial strain	Indole test	Siderophore production test	Phosphate solubilization test	Methyl red test	Vogesproskauer test	Catalase test	Dual culture	HCN production test
RPBHI1	-	-	+	-	+	+	-	+
RPBHI2	-	-	-	-	-	+	-	-
RPBHI3	-	-	-	+	+	+	+	+
RPBHI4	-	-	+	-	+	+	-	-
RPBHI5	-	-	-	+	-	-	+	-
RPBHI6	-	-	+	-	+	+	+	-

In the current study 72.72% of endophytic and 50% of rhizoplane cultures showed significant antifungal activity against phytophthora. Isolates ENBHI1, ENBHI3, ENBHI4, ENBHI5, ENBHI7, ENBHI8, ENBHI9, ENBHI10, RPBHI1, RPBHI3, RPBHI4 and RPBHI5 are very efficient in suppressing the growth of Phytophthora pathogens. Phytophthora species are considered to be soil borne pathogens responsible for many plant diseases. These pathogens were used to test the antifungal activity of isolates with dual culture method (**Image 1 and 2).**

Image 1 shows the control plate of phytophthora and Image 2 shows how its growth has been inhibited by one of the endophytic isolate ENBHI9. 53% of endophytic and 83.3% of rhizoplane cultures are catalase positive. 27.27% of endophytic and 33.33% of rhizoplane bacteria are HCN positive. Tests conducted to check for growth promoting characteristics were IAA test, siderophore production test and phosphate solubilisation test. One endophytic isolate has shown positive indole production test and none of rhizoplane bacteria were positive for IAA test. 3 strains of endophytic and 3 strains of rhizoplane bacteria are positive for phosphate solubilisation. 2 of endophytic isolates are showing positive result for qualitative siderophore production test which was determined through chrome azurol test.


Image 1: Phytophthora control plate


Image 2: Endophytic isolate ENBHI9 streaked on both sides of Phytophthora pathogen

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Discussion:

Endophytic bacteria have been reported to be isolated from various medicinal plants like Capparissinaica vell **(Bhagat *et al.*, 2014)**, Oscumum sanctum **(Tiwari *et al.*, 2010),** catharanthus roseus, Mentha arvensis, Stevia rebaudiana **(Anjum *et al.*, 2015),** and Achyranthus aspera **(Khaidem, 2017).** To our knowledge this current study is the first to isolate bacteria from *H. indicus*. The role of endophytic bacteria in growth, development and fighting phytopathogens are well documented **(Jasim *et al.*, 2014; Reiter *et al.*, 2002)**.

In the current study, different growth media like Tryptone soya agar, Kings B, and Rovira media were used to isolate endophytic and rhizoplane bacteria from plant root. Different medias were used to get isolates which are non culturable on single media. Total numbers of endophytic bacteria isolated were 11 and rhizoplane bacteria were 6. The role of these organisms in growth, development and suppressing phytopathogens are well documented. In the current study we conducted biochemical tests like siderophore production test, Indole acetic acid test, and phosphate solubilization test in order to determine the growth promoting ability of these organisms and antifungal activity test, Hydrogen cyanide test and catalase test to check the antimicrobial activity of bacterial isolates. We found that the 3 of endophytic and 1 of rhizoplane bacteria are positive for all 3 tests (HCN, catalase and antifungal activity) indicating the significant pathogen suppressing activity. In addition 8 of endophytic and 3 of rhizoplane bacterial isolates are showing antifungal activity. This was determined by dual culture method in which phytophthora fungal pathogen was used. Phytophthora species are considered to be soil borne pathogens which cause plant diseases **(Shouan zhang, 2010).** Compared to control, plate of phytophthora, the plates with bacterial isolates streaked on either side of fungal pathogen showed significant reduction in the growth of pathogen. This is shown in the **image 1 and 2**. Potential of these organisms to suppress soil borne pathogen has been earlier examined in potato tubers and cocoa plant and results by other authors have been promising. **(Melnick *et al.*, 2008; Sturz *et al.*, 1999).**

These organisms produce other antimicrobial compounds like Hydrogen cyanide and enzyme like catalase which are toxic to bacterial and fungal pathogens **(Deshwal *et al.*, 2013).** Considering the results of above tests significant pathogen suppressing ability of endophytic and rhizoplane bacteria isolated from *H. indicus* can be concluded.

Indole acetic acid (IAA) is one of the main naturally occurring auxin (a plant growth hormone). IAA controls cell elongation, division, and tissue differentiation resulting in plant growth and development **(Jasim *et al.*, 2014)**. One endophytic isolate has shown positive indole production test. Plants are often unable to utilize the insoluble phosphate which is present in soil. Certain bacteria are capable of solubilizing the immobile phosphate through the production of organic acids and phosphatase enzyme and thus make it available for plant. Appearance of halo zone around the bacterial colonies grown on the media containing tri calcium phosphate indicates the phosphate solubilizing ability of bacteria. Three strains of endophytic and three strains of rhizoplane bacteria are positive for phosphate solubilization.

Two endophytic isolates are showing positive result for qualitative siderophore production test. Siderophore, an iron chelating molecule aids plant in iron uptake from soil and thus making it unavailable to pathogens **(Khaidem *et al.*, 2017).**

H. indicus is a medicinal plant which is being over exploited for its medicinal values and is now considered as rare and endangered plant **(Subbaiyan *et al.*, 2014).** Endophytes associated with the plant could be one of the ways to increase the plant population. Our current study has shown the growth promoting properties as well as its ability to enhance disease resistance in the plant. It is suggested that endophytes have symbiotic relationship with their host and acts as natural biocontrol agents to protect plants from phytopathogens. As a next step to this study, we wish to apply endophytic and rhizoplane bacteria isolated from *H. indicus* directly to host plant to see if it is actually enhancing the growth of the plant and to study if the organisms act as bio control agents.

Conclusion:

From the foregoing, it can be concluded that *H. Indicus* – a rare and endangered plant, has the ability to produce endophytic and rhizoplane bacteria. Total of 11 endophytes and 6 rhizoplane bacteria were isolated. Eight among 11endophytes and 3 among 6 rhizoplane cultures showed antibacterial property. In addition, 3 of 11 endophytes and 3 of 6 rhizoplane cultures showed significant plant growth promoting properties. This

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indicates the potential use of these organisms as bio control agents.

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DETERMINATION OF QUALITATIVE EFFECTS ON SOIL BY AQUEOUS EXTRACT OF *Ocimum gratissimum*

Research article

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ABSTRACT

Ocimum gratissimum is an aromatic plant rich in essential oils. The aim of the study was to check the effects of the aqueous leaf extract on soil fertility when treated on fenugreek plants. The fenugreek plant was grown in two-paper pot labeled as CONTROL, treated with just water and SAMPLE, treated with aqueous extracted. It was grown for 25 to 30 days. After harvesting, the soil of both CONTROL and SAMPLE was tested for NPK and pH. The soil of SAMPLE showed good NPK and pH content when compared CONTROL, which is very important for growth of any crop.

INTRODUCTION

O. gratissimum is a shrub up to 1.9 m in height with stems that are branched. The leaves measure up to 10 x 5cm, and are ovate to ovate-lanceolate, sub-acuminate to acuminate at apex, cuneate and decurrent at base with a coarsely crenate, serrate margin, pubscent and dotted on both the sides. The leaves show the presence of covering and glandular trichomes. Stomata are rare or absent on the upper surface while they are present on the lower surface. Ordinary trichomes are few, while the long ones up to 6-celled are present on the margins mostly; the short ones that are 2 celled, are mostly found on the lamina. Petioles are up to 6 cm long and racemes up to 18 cm long. The peduncles are densely pubescent. Calyx is upto 5 mm long, campanulate and 5-7 mm long, greenish- white to greenish-yellow in colour **(K.S. Prabhu *et al.*, 2009).**

It is reported that the presence of important phytochemicals are alkaloids, tannins, flavonoids and phenolic compounds. *Ocimum gratissimum* (OG) is grown for the essential oils in its leaves and stems **(Afolabi C. Akinmoladun *et al.*, 2007).**

Trigonella foenum-graecum (Fenugreek) plant reported to be used for blood lipids and sugar decreasing in diabetic and non-diabetic peoples and has antioxidant and antibacterial activity. This plant use in therapy atherosclerosis, rheumatism, sugar lowering, blood lipids lowering, appetizer and contain antioxidant activity **(Rashmi Yadav *et al.*, 2014).**

Fig 1: *Ocimum gratissimum* (OG)

Fenugreek requires well-drained, good soil of medium texture. Tolerated pH range is 5.3 to 8.2. Seeds are sown directly in the garden in spring, as soon as the danger of frost is past. The plant reaches a height of 0.3 to 0.8 meters and has trifoliate leaves. As a leguminous plant, fenugreek needs little if any nitrogen fertilizer, and the plant can enrich soils with nitrogen. **(Mullaicharam AR *et al.*, 2013).**

Fenugreek seeds were chosen since it was easy to handle, observation and less time consuming. The present experiment was conducting in Harihar Tq,

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Davangere district. The leaves of OG for extraction process were procured from the surrounding area of Harihar. The average atmospheric temperature is around 20^0C to 30^0C and humidity is 76% to 79% through out the study. The purpose of this study is to understand the effects of aqueous leaf extract as fertilizer in growth and development of crops.

MATERIALS AND METHOD: (Nice soil testing kit, 2008)

DETERMINATION OF SOIL – pH

Reagents:

1. pH Reagent – 1 (pH-1)
2. pH Reagent – 2 (pH-2)
3. Decolourizer (D-1) & pH color chart (Chart No – 1)

Test Method

1. Measure 10 cc of soil and transfer into soil mixing tube.
2. Add 25 mL of pH Reagent–1 (pH–1) into the soil and shake well for 5 minutes, then add a pinch of Decolourizer (D-1) into the soil mixture, again shake well. Then filter into the color-developing bottle by using a funnel and filter paper.
3. To the clear filtrate, add 4 – 5 drops of pH – Reagent -2 (pH – 2) and mix well. Wait 2-3 minutes for color to develop. The color that forms is compared with the pH color chart (chart No.1).

ESTIMATION OF AVAILABLE NITROGEN IN SOIL

Reagents:

1. Nitrogen Reagent – 1 (N – 1)
2. Nitrogen Reagent – 2 (N - 2)
3. Decolourizer (D-1) & Nitrogen color chart (Chart No – 2)

Test Method

1. Measure 5 cc of soil and transfer into soil mixing tube.
2. Add 25 mL of Nitrogen Reagent–1 into the soil and shake for 5 - 10 minutes, then add a pinch of Decolourizer (D-1) into the soil mixture, again shake well. Then filter into the color-developing bottle by using a funnel and filter paper.
3. To the clear filtrate, add 2 drops of Nitrogen reagent-2 (N–2) and mix well. Wait 1-2 minutes for color to develop. The color that forms are compared with the Nitrogen color chart (chart No. 2) and record as Low (L1 & L2), Medium (M1 & M2) or High (H1 & H2). Discard the solution and wash all the tubes well.

ESTIMATION OF AVAILABLE PHOSPHOROUS IN SOIL

Reagents:

1. Phosphorous Reagent – 1 (P – 1)
2. Phosphorous Reagent – 2 (P - 2)
3. Decolourizer (D-1) & Phosphorous color chart (Chart No – 3)

Test Method

1. Measure 5 cc of soil and transfer into soil mixing tube.
2. Add 25 mL of Phosphorous Reagent – 1 (P – 1) into the soil and shake for 15 minutes. Then add a pinch of Decolourizer (D-1) into the soil mixture, again shake well, and then filter into the color-developing bottle (No – 3) by using a funnel and filter paper.
3. To the clear filtrate, add 2ml of Phosphorous reagent -2 (P – 2) and mix well. Wait 1-2 minutes for color to develop. The color that forms are compared with the Phosphorous color chart (chart No. 3) and record as Low (L1 & L2), Medium (M1 & M2) or High (H1 & H2). Discard the solution and wash all the tubes well.

ESTIMATION OF AVAILABLE POTASSIUM IN SOIL

Reagents:

1. Potassium Reagent – 1 (K – 1)
2. Potassium Reagent – 2 (K - 2)
3. Decolourizer (D-1) & Phosphorous color chart (Chart No – 4)

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Test Method

1. Measure 5 cc of soil and transfer into soil mixing tube.
2. Add 25 mL of Potassium Reagent – 1 (K – 1) into the soil and shake for 10-15 minutes. Then add a pinch of Decolourizer (D-1) into the soil mixture, again mix well, and then filter into the color-developing bottle (No – 3) by using a funnel and filter paper.
3. To the clear filtrate, add 1mL of Potassium reagent -2 (K – 2) and mix well. Wait 1-2 minutes for color to develop. The color that a form is compared with the Potassium color chart (chart No. 4) and record as Low (L1 & L2), Medium (M1 & M2) or High (H1 & H2). Discard the solution and wash all the tube

Result:

- Soil falling between pH – 6.5 to 8.0 is generally suitable for most of the common crops. The soil in the SAMPLE showed the medium alkaline which is recommended for good growth of plants where as soil of CONTROL falls in very strongly alkaline, which is not good for growth of plants.

Table 1: Nitrogen content

Amount of available Nitrogen in Soil		Approximate quantity of available Nitrogen present in Kg/Acre
Low (<100 Kg/Acre)	L1	<50 Kg/Acre
	L2	50 – 99 Kg/Acre
	M1	100 – 150 Kg/Acre
	M2	151 – 200 Kg/Acre
High (>200 Kg/Acre)	H1	201 – 300 Kg/Acre
	H2	>300 Kg/Acre

Table 2: Phosphorus content

Amount of available Phosphorous in Soil		Approximate quantity of available Phosphorous present in Kg/Acre
Low (<4 Kg/Acre)	L1	<1 Kg/Acre
	L2	1 – 3 Kg/Acre
Medium (4-10 Kg/Acre)	M1	4 – 7 Kg/Acre
	M2	8 – 10 Kg/Acre
High (>10 Kg/Acre)	H1	11 – 15 Kg/Acre
	H2	>15 Kg/Acre

Table 3: Potassium content

Amount of available Potassium in Soil		Approximate quantity of available Potassium present in Kg/Acre
Low (<50 Kg/Acre)	L1	<25 Kg/Acre
	L2	25 – 49 Kg/Acre
Medium (50-120 Kg/Acre)	M1	50 – 80 Kg/Acre
	M2	81 – 120 Kg/Acre
High (>120 Kg/Acre)	H1	121 – 150 Kg/Acre
	H2	>150 Kg/Acre

Table 4: Availability of N, P, K in CONTROL and SAMPLE soil

	CONTROL	SAMPLE
pH	pH falls between 8.5-9.0.	pH falls between 7.0-7.5
Nitrogen,N	L1= <50kg/acre	M1= 100-150kg/acre
Phosprous,P	M2= 8-10kg/acre	H1=11-15kg/acre
Potassium,K	H1=121-150kg/acre	M2=81-120kg/acre

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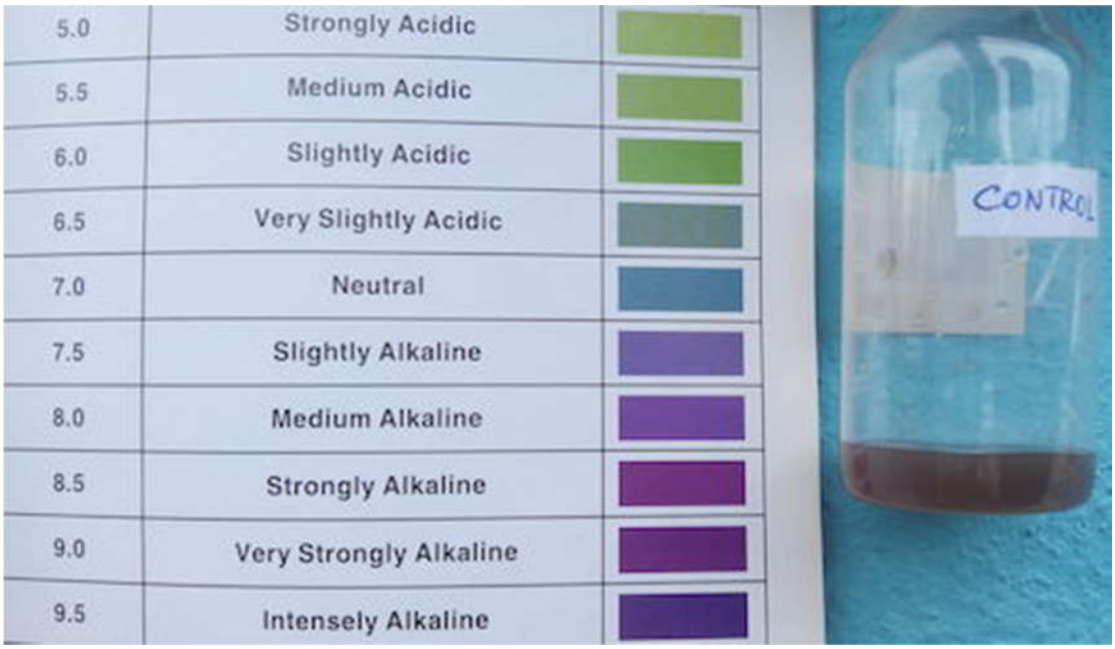

Figure 2: In determination of pH of soil in control, the pH range falls in between strongly alkaline and very strong alkaline

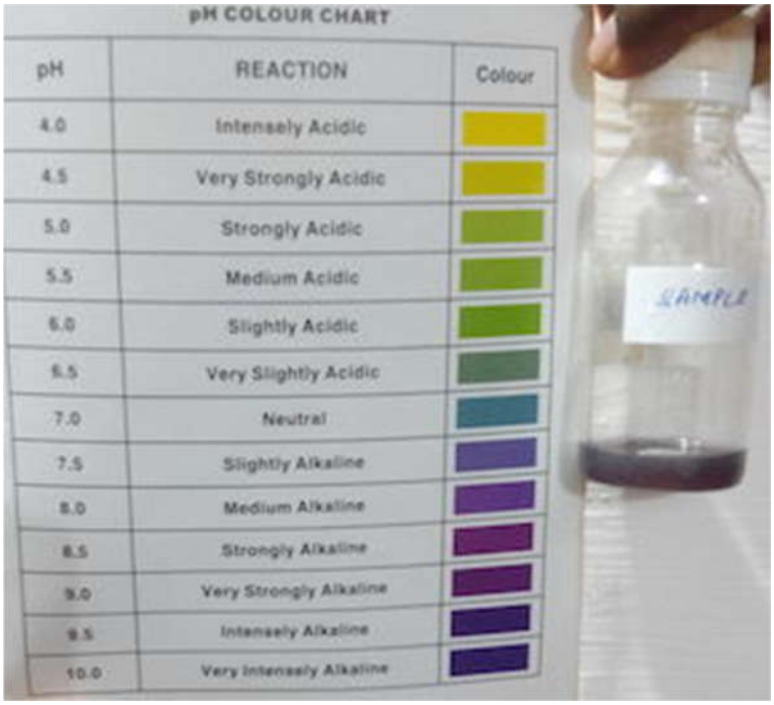

Figure 3: In determination of pH of soil in sample, the pH range falls in slightly alkaline in SAMPLE

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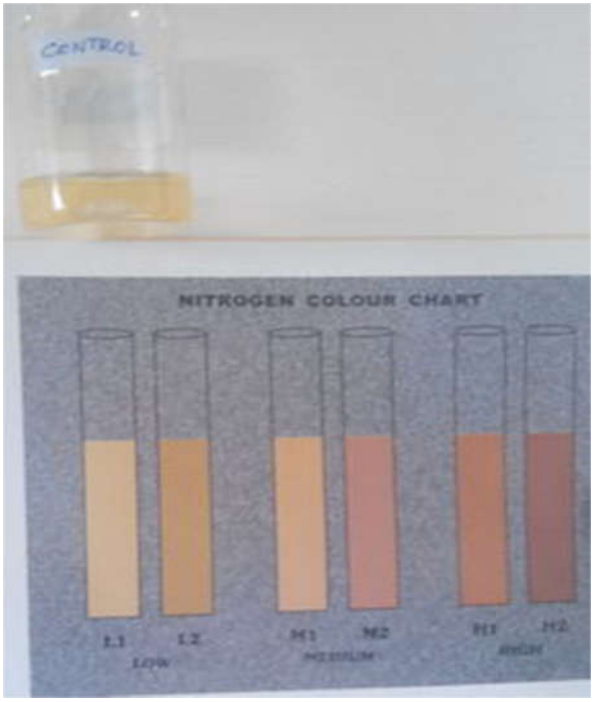

Figure 4: In determination of nitrogen of soil in control, the color range falls in L1 for control

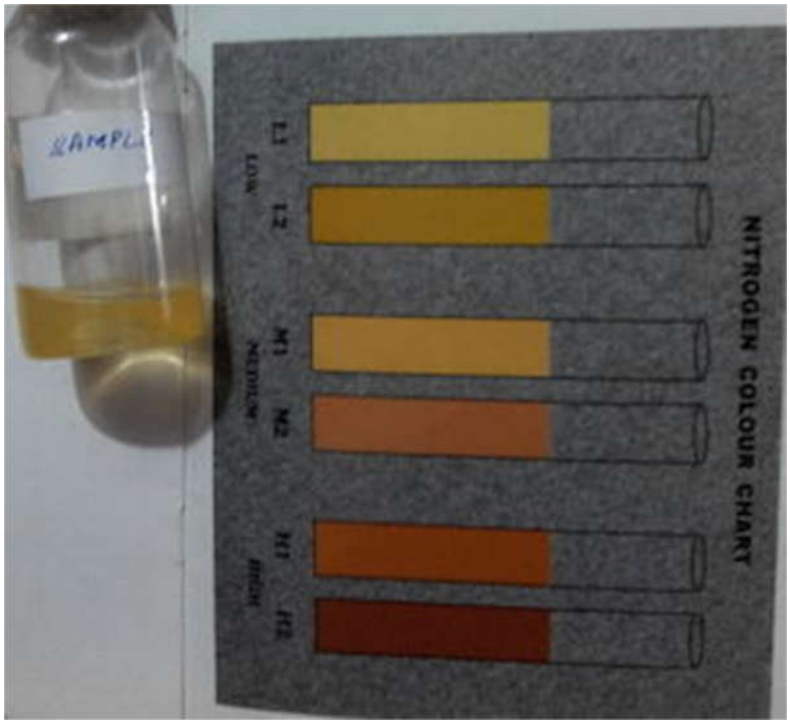

Figure 5: In determination of nitrogen content of soil in sample, the color range falls in M1 for sample

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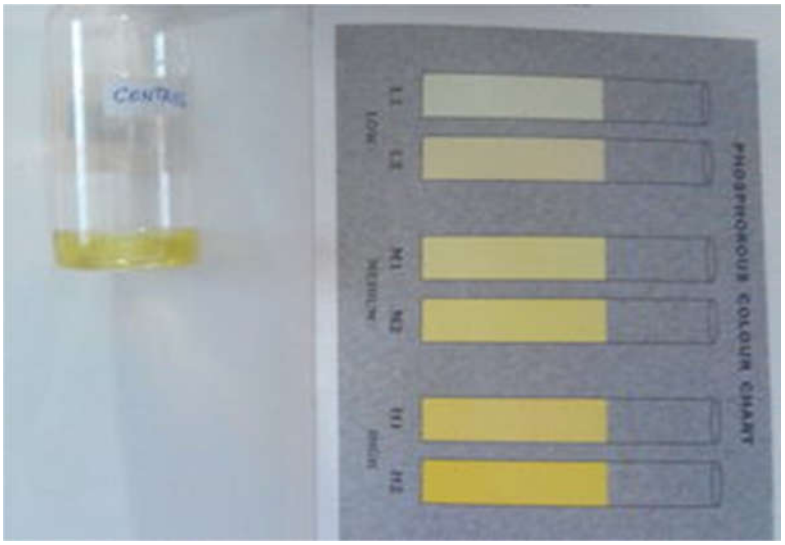

Figure 6: In determination of phosprous content of soil in control, the color range falls in M1 for control

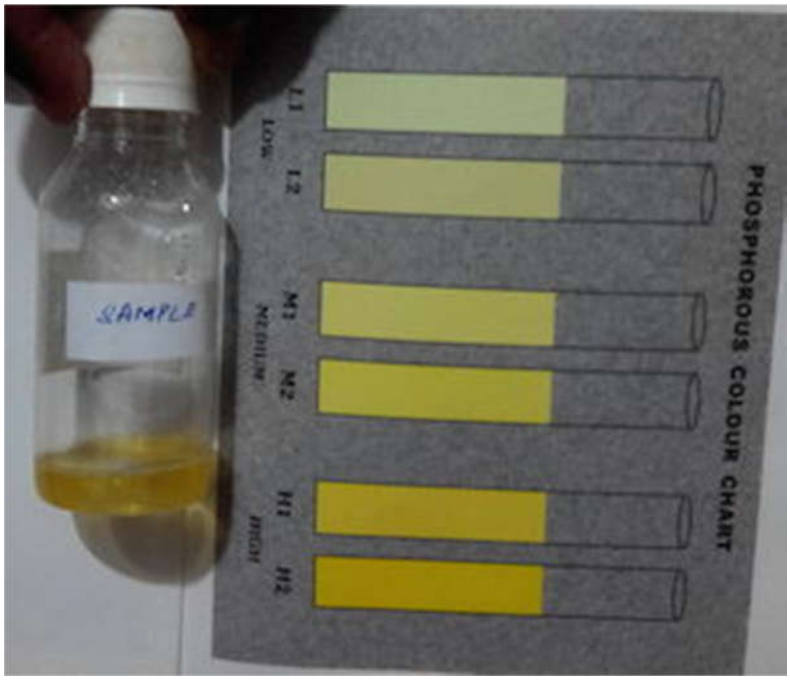

Figure 7: In determination of phosprous content of soil in sample, the color range falls in H1 for sample

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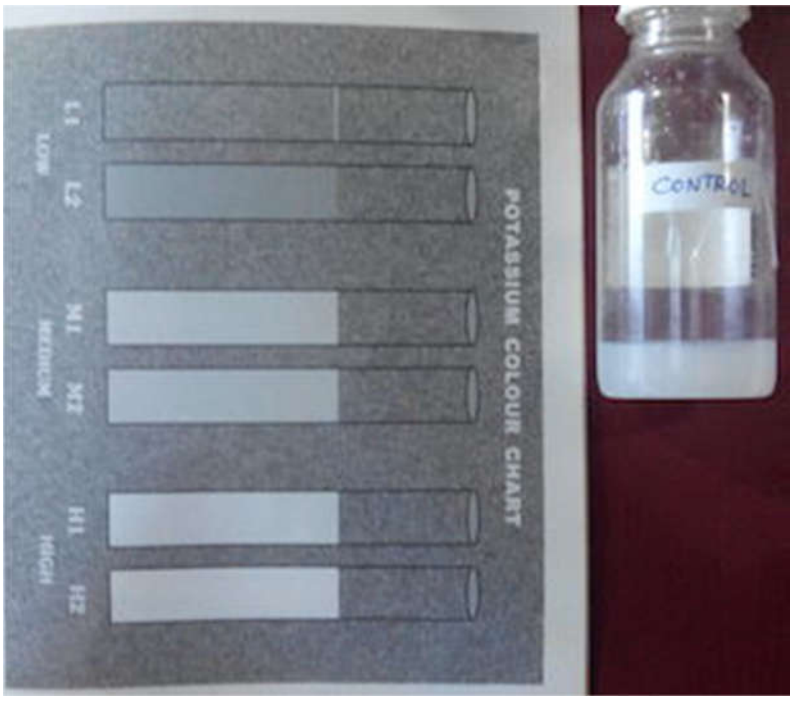

Figure 8: Indetermination of potassium content of soil in control, the color range falls in M2 for sample

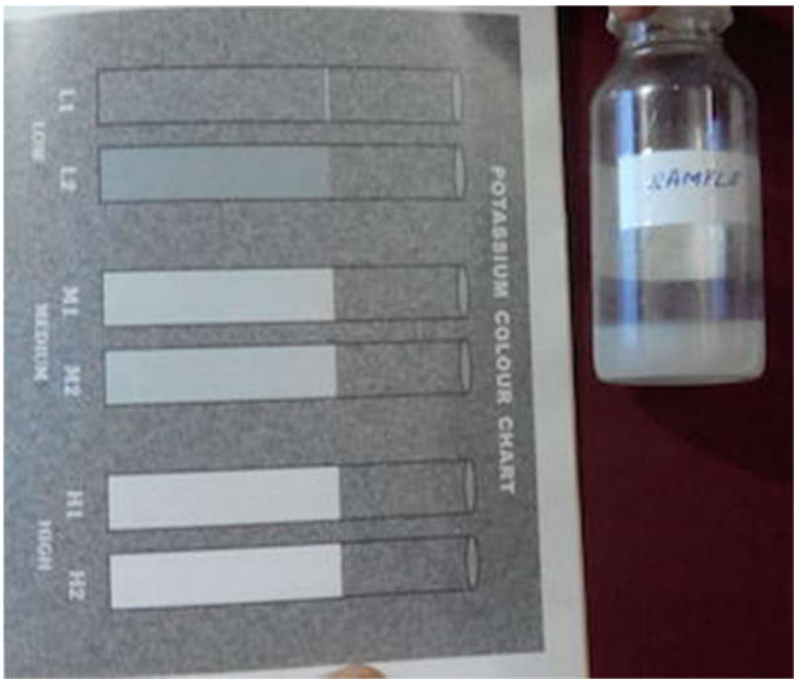

Figure 9: In determination of potassium content of soil in sample, the color range falls in H2 for sample

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DISCUSSION

From the below discussion we will come to know that the quality of the soil has increased in SAMPLE due to the addition of aqueous extract of *O.gratissimum* which lead to the good growth of the plants where as CONTROL showed comparatively poor value of NPK and pH in the soil.

pH: the soil seems to be alkaline in CONTROL (fig 2) and the soil in SAMPLE (fig 3) falls in the recommended pH for good growth of crops.

Nitrogen: The soil falls in L1 range for CONTROL (fig 4) which means availability of nitrogen is very less in CONTROL, 25% more nitrogen to be added with the recommended dosage. Soil of SAMPLE falls in range M1 (fig 5) that means the availability of nitrogen is good in the SAMPLE, recommended dosage mentioned in the fertilizer package is added (table 1).

Phosphorus: The soil falls in M2 range for CONTROL (fig 6) which means availability of phosphorus is medium in CONTROL, recommended dosage mentioned in the fertilizer package is added. Soil of SAMPLE falls in range H1 (fig 7) which means the availability of phosphorus is more, 25% less phosphorus to be added than the recommended dosage (table 2).

Potassium: soil of CONTROL falls in range H1 (fig 8) which means the availability of potassium is more, 25% less potassium to be added than the recommended dosage. The soil of SAMPLE falls in M2 range (fig 9) which means availability of potassium is medium in SAMPLE, recommended dosage mentioned in the fertilizer package is added (table 3).

CONCLUSION

From this study we can conclude that the aqueous leaf extract treated soil showed a good content of NPH and in pH in SAMPLE in comparison to the soil of CONTROL. It can be concluded that *O.gratissimum* can be used as fertilizer in the appropriate manner. Further studies has to be conducted and can be brought into the usable form for the improvement of the crops.

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