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# Biological potential of *Ascophyllum nodosum* extract on rhizobial diversity in nodules of mothbean *Vigna aconitifolia* Jacq. via Amplified Ribosomal DNA Restriction Analysis

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## Abstract

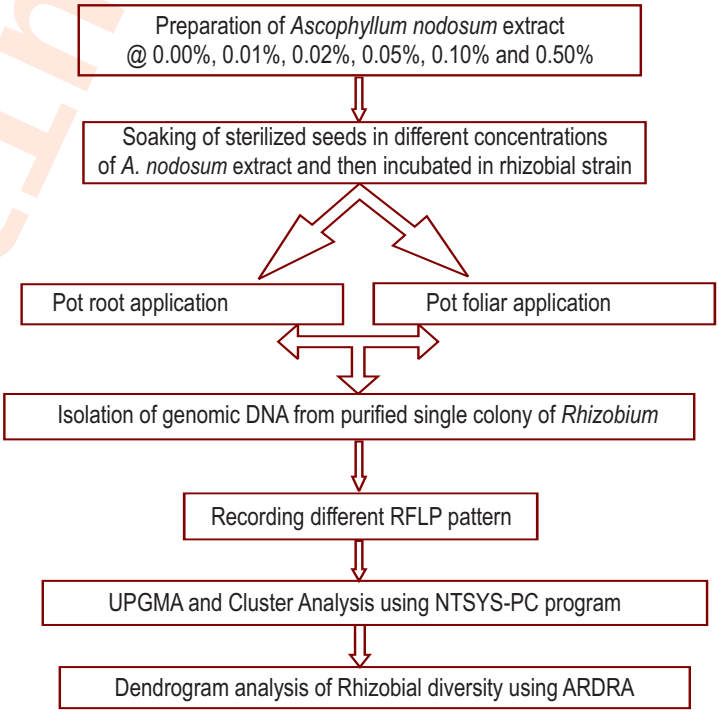
**Aim:** The aim of this study was to use *Ascophyllum nodosum* for potentially increasing the growth and rhizobial diversity in nodulating rhizobia in *Vigna aconitifolia*.

**Methodology:** Different concentrations of *Ascophyllum nodosum* extracts (0.01%, 0.02%, 0.05%, 0.10% and 0.50%) were applied via foliar spray and on roots of *Vigna aconitifolia*. Growth characteristics and Amplified Ribosomal DNA Restriction Analysis were conducted to detect the morphological and molecular changes in rhizobial diversity. The restriction profiles thus obtained were used to study the rhizobial communities via Cluster analysis and Dendrogram using NTSYS-PC program and UPGMA constructed.

**Results:** Roots treated with 0.05% *Ascophyllum nodosum* extract showed best growth of plants. This concentration not only proved best for the aggregation of nodules but also for obtaining enormous rhizobial diversity.

**Interpretation:** *Ascophyllum nodosum* is a modern, cheap, non-toxic natural biofertilizer and Amplified Ribosomal DNA Restriction Analysis represents a favorable alternative to culture dependent method for assessing rhizobial diversity in nodulating bacteria.

**Key words:** ARDRA, *Ascophyllum nodosum*, *Rhizobium, Vigna aconitifolia*

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## Introduction

Pulses form an important nutritive supplement of human diet in India (Verma *et al.,* 2017a).Being a rich source of protein, it is known as "poor men's meat" because of its low cost as compared to animal proteins (Vietmeyer, 1986). In addition to high protein content, legumes are low in fat content. A good amount of dietary fibres and many micronutrients in legumes contribute to its nutritional properties (Rungruangmaitree *et al.,* 2017). *Vigna acontilifolia* commonly known as moth or Turkish gram is a drought-resistant legume, also growing in arid and semi-arid regions of India and Pakistan. Rajasthan, is the main moth bean producing state and contributes about 86% production in the country. It is also used to reduce fever and the roots used are as narcotic (Chunekar and Pandey, 1998; Watt *et al.,* 1889). More than 1000 accessions of *V. acontilifolia* are available in National Bureau of Plant Genetic Resources (NBPGR), New Delhi, India, out of which RMO 40 and RMO 225, cultivars are mainly grown in India.

Most legumes have the capacity to formulate a symbiotic relationship with $N_2$-fixing soil microorganisms rhizobacteria, broadly known as Rhizobia in the family Rhizobiaceae e.g., *Bradyrhizobium, Rhizobium, Allorhizobium, Mesorhizobium, Azorhizobium,* or *Sinorhizobium* (Velazquez *et al.,* 2010). Rhizobia are Gram-negative, motile, non-sporulating rods (Long, 1996), genetically diverse and physiologically heterogenous group of bacteria (Somasegaran and Hoben, 1994). In the rhizosphere of legumes, rhizobia are very important part of soil micro-flora (Allen and Allen, 1981; Somasegaran and Hoben, 1994). Rhizobia play a significant role in nitrogen acquisition through symbiotic nitrogen fixation in a broad variety of leguminous plants (Fujihara *et al.,* 2002). Sylvie and Patrick (2010) reported that the application of *Rhizobium* in *Phaseolus vulgaris* significantly improved the number of pods per plant, number of seeds per plant, seed weight and seed yield. Leguminous crops are widely distributed; however, in some places the soil lack rhizobial population (Brockwell *et al.,* 1995).

Seaweed extract is a new generation natural organic fertilizer with high nutritive value. It has been found to enhance the germination, nutritional value of seeds of many crops (Verma *et al.,* 2017b). Fertilizers derived from seaweeds are known as Seaweed Liquid Fertilizers. The seaweed extract contains all the essential components beneficial for the growth, development, defence against diseases (Wu and lin, 2000; Verma *et al.,* 2020). Seaweed fertilizer is an effective alternative to chemical fertilizer as it is easily absorbed by plants and has no harmful effects on the ecosystem (Sathya *et al.,* 2010). Jae-Suk Choi RIS *et al.* (2011) reported that methanolic extracts of three species of seaweed could be used as therapeutic agent. Now a day, the most important seaweed that plays a very important role in agriculture is *Ascophyllum nodosum*. *Ascophyllum nodosum* is a brown aquatic alga (Phaeophyceae) that grows along low lying and backwaters area and dominates the rocky intertidal zones of the Atlantic shores of Nova Scotia and New Brunswick, Canada (Ugarte *et al.,* 2010). Application of seaweed extract triggers the growth of important soil microbes and secretion of soil conditioning substances by these microbes. Alam *et al.* (2013) reported the positive effects of *Ascophyllum nodosum* extract on strawberries, as well as benefit to soil microbial community. Seaweed manure increases the moisture holding capacity and soil fertility by adequate supplying trace elements to improving the soil structure (Dhargalkar and Neelam Pereira, 2005). Arun et al. (2014) observed that application of seaweed extract not only improve the growth of *Abelmoschus esculentus* and *Solanum Lycopersicumbut* also improve the microbial diversity. In the present study *Ascophyllum nodosum* Extract (ANE) applied for studying the improvement in growth parameters of *Vigna acontifolia*. Further experiments were conducted to determine the nodules quality and quantity. The molecular changes in rhizobial diversity in nodulating bacterial was also carried out using Amplified Ribosomal DNA Restriction Analysis (ARD RA) and the cluster analysis was done.

## Materials and Methods

**Experimental design:** The *Ascophyllum nodosum* (L.) Le Jolis, (Seaweed) (Trade name: Biovita) extract (ANE) was purchased from PI industries, Rajasthan. Seeds of *Vigna aconitifolia* (RMO 225) were purchased from Rajasthan and the rhizobial strains of *V. acontilifolia* were procured from the Department of Microbiology, CCSHAU, Hisar, India. Two experiments *viz.,* Pot Root Application and Pot Foliar Application were carried out. Five different concentrations of *Ascophyllum nodosum* extract (@ 0.01%, 0.02%, 0.05%, 0.10%, and 0.50%) and control (without extract) were prepared. Plastic pots measuring 30 x 30 cm were filled with 4.0 kg of sterilized river sand, autoclaved at 121°C for 1 hr.

The seeds were surface sterilized with 0.1% mercuric chloride for 3-4 min, washed 5-6 times with sterilized double distilled water. For pot root application, sterilized seeds were soaked in different concentrations of seaweed extract for 12 hrs and then the seeds were inoculated with rhizobial strain ($10^9$ cells) for 1 hr. Nitrogen free Slogar's solution was added every second evening alternating with water. A 5 ml of each seaweed extract was applied at a regular interval of 15 days to the roots *V. acontilifolia*. Whereas, for pot foliar application sterilized seeds were soaked in rhizobial strain directly without soaking in the seaweed extract and grown in similar manner as pot root application, except 5.0 ml of different concentration of *A. nodosum* extract were sprayed to foliar parts of plants. Each treatment was carried out in triplicate and three plants per pot were kept for further study. The number of nodules per plant in each treatment was recorded after uprooting the plants on day 80.

**Isolation of rhizobia:** The roots from different ANE treated plants from PRA and PFA experiments were harvested. The nodules from roots were washed thoroughly, surface sterilized with 0.1% $HgCl_2$ (2-3 minutes) and dipped in 95% ethanol solution and then finally washed (6-7 times) with sterilized distilled water. The surface sterilized nodules were crushed with sterilized glass rod in minimal amount of sterilized water. A loopful of milky suspension obtained by crushing the nodules were streaked on sterilized Yeast Extract Mannitol Agar (Fred *et al.,* 1932) medium plates. The plates were then incubated at 30 ºC for 24 hr. *Rhizobium* cultures were streaked repeatedly on fresh YEMA plates to get a single purified colony. Finally, the purified single rhizobial isolates were cultured on fresh YEMA. After that the rhizobia were streaked on Tryptone Yeast Agar (Bringer, 1974) medium and then inoculated on TY broth for 24 hrs for further molecular studies.

**Isolation of Genomic DNA:** Genomic DNA was isolated following the method of Ausubel *et al.* (2002) with some modification. Bacterial strains isolated from all the treatments were cultured in TY medium for 48 hr. 1.5 ml of each culture was centrifuged for 5 min at 12,000 rpm till a compact pellet was formed. The pellet was resuspended in 570 µl TE buffer, 30 µl of 10 % SDS and 4 µl of 20 mg $ml^{-1}$ proteinase K. All components were added to make a final volume of 100 µg $ml^{-1}$ proteinase K in 0.5 % SDS. After proper mixing, these were again incubated for 3 hr at 37 ºC in a water bath mixed with 100 µl NaCl. To this mixture, CTAB/NaCl solution was added and mixed thoroughly and incubated for 10 min at 65°C in a water bath. Chloroform/isoamyl alcohol (24:1) of approximately equal volume was added and spinned at 12,000 rpm for 10 min.

To equal volume of supernatant, phenol/ chloroform/ isoamyl alcohol (25:24:1) was added. In a fresh tube, the aqueous phase was taken and the supernatant was mixed with two third volume of isopropanol (kept at 4°C) to precipitate the nucleic acids. The tube was shaken to and forth till a stringy white DNA precipitate was clearly visible and tubes were incubated overnight at -4°C. The following DNA was pellet by centrifugation for 15 min at rpm 12,000. After discarding the supernatant, the pellet of each treatment was washed with 200 µl of 70 % ethanol (kept at -4°C) and centrifuged at 12,000 rpm for 5 min. Pellets from each sample were dissolved by tapping in TE buffer, incubated for 10 min at 65°C, if required tapping was done again. Finally, the DNA present in the buffer solution was stored in a deep freezer.

**Quantification of Genomic DNA:** Genomic DNA was quantified by reading the absorbance at 260 nm and 280 nm wavelength. The amount of DNA was calculated by using the relationship that O.D. of 1.0 corresponds to 50 µg $ml^{-1}$. The purity of DNA was checked by measuring the ratio of A260/A280; 1.5 - 1.8 values is for pure DNA. Purity of DNA was also checked by observing bands on 0.8 % agarose gel.

**Amplification of 16S rDNA sequences by Amplified Ribosomal DNA Restriction Analysis:** Amplification of 16S rDNA sequences was carried out by polymerase chain reaction using a thermal cycler MJ Research, USA (Lukow *et al.,* 2000). The forward primer BAC 27F (5'- AGA GTT TGA TCC TGG CTC AG - 3') and reverse primer 1378R (5'- CGG TGT GTA CAA GGC CCG GGA ACG - 3') allow the amplification of 16S rDNA gene sequence that present in rhizobial DNA. The genomic DNA was cut with individual restriction enzyme (*Hae III* and *Msp I*) separately. The fragments thus obtained were separated on 2% agarose gel. Size of amplified 16S rDNA fragments was analyzed by using 100-1000 bp DNA ladder (medium range, Quigen) and different RFLP pattern were recorded.

**Scoring and analyses:** The size of each obtained band was compared with the standard marker (100-1000 bp DNA ladder) and the profiles of the isolates were prepared. Only reproducible bands were scored and analyzed. Matrix was prepared from 0-1 on the basis of presence or absence of a particular band. By following Sim Qual Coefficient, Similarity matrices were made and analyzed by UPGMA (Unweighted Pair Grouping with Mathematic Average) cluster analysis using NTSYS-PC program (version 2.1: Exeter Software, Setauket, N.Y.) (Rohlf, 1998). UPGMA Dendrograms were constructed from the genetic similarity between different *rhizobia*.

## Results and Discussion

*Rhizobium* interaction plays an active role in plant growth. This experiment was conducted to understand the role of bioactive components of *Ascophyllum nodosum* extract on the growth and nodule formation. *Ascophyllum nodosum* extract was applied by two different methods namely, pot root application and pot foliar application to explore the possibility of application process to mitigate the logistic costs.The growth was recorded up to 75 days, at an interval of 15 days in both pot foliar application and pot root application of 0.00%, 0.01%, 0.02%, 0.05% and 0.10% and 0.50% of *Ascophyllum nodosum* extract. It was observed that root application of extracts showed significant growth and increase in leaf size (Fig. 1A, B). The 0.05% of *Ascophyllum nodosum* extract was the best for growth as well as for procuring nodules(Fig. 2A, B). This concentration was superior nodule quality in both foliar and root application of *Ascophyllum nodosum* extract experiments. The findings of this study is agreement with the previous studies which showed that the application of seaweed extract increases the growth of soil microbes and soil fertility which was attributed to the bioactive compounds present in the seaweed extract (Alam *et al.,* 2013). *Ascophyllum nodosum* positively affected the nodule formation in legume, Alfalfa (Khan *et al.,* 2012; Khan *et al.,* 2013; Zhai, 2012). Verma *et al.* (2019) observed that tyrosinase inhibition activity and photosynthetic pigments accumulation also increased in 0.05% *Ascophyllum nodosum* extract treated sprouts of *Vigna aconitifolia*. Higher concentrations of *Ascophyllum nodosum*

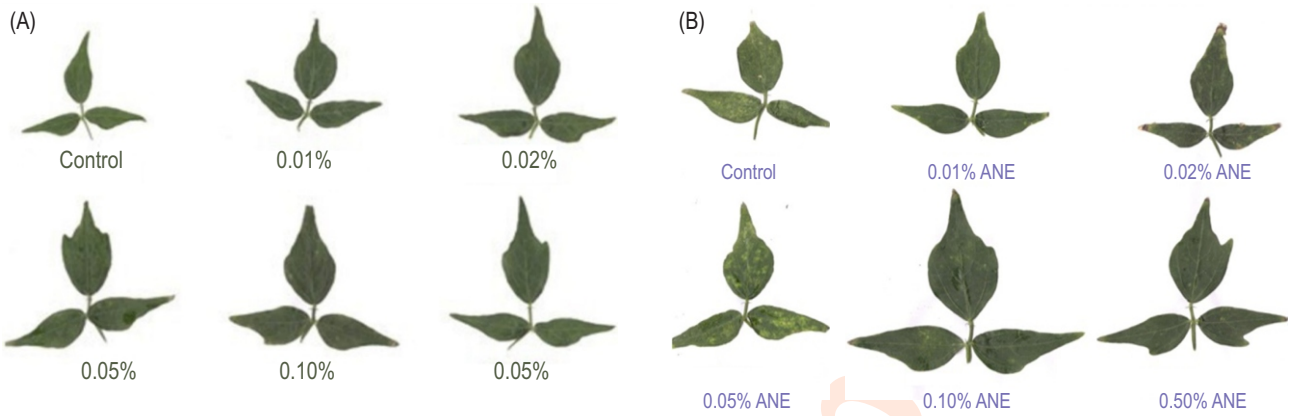


**Fig. 1 :** Leaf area of *Vigna acontifolia* after one month treatment with different concentrations of *A. nodosum* extracts in (A) Pot foliar application and (B) Pot root application.

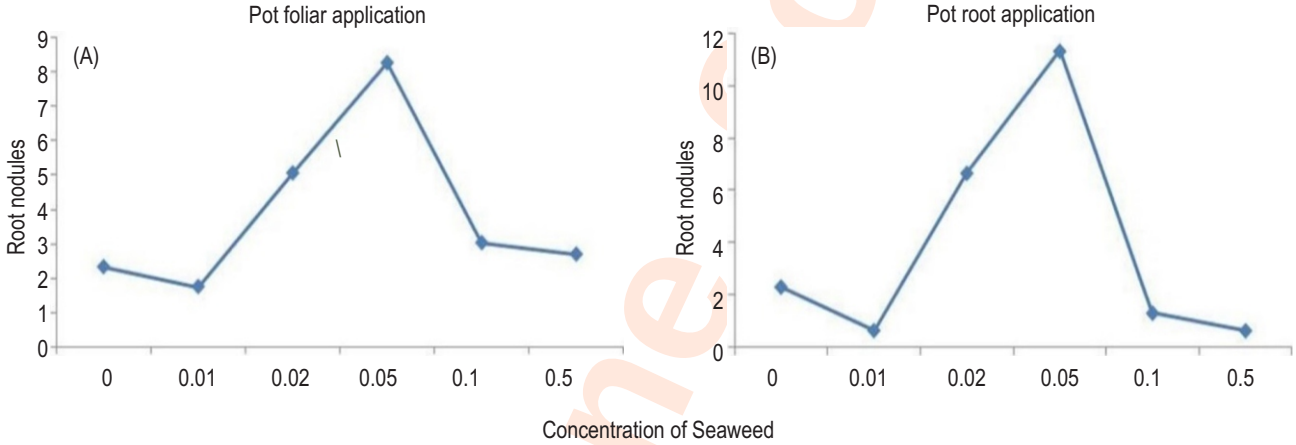


**Fig. 2 :** Effect of different concentrations of *Ascophyllum nodosum* extract on the number of nodules in *Vigna aconitifolia* in (A) Pot folior application and (B) Pot root appliation .

extract may lead to loss of chloroplast integrity due to browning of cotyledons (Wu and Lin, 2000). All the *Vigna aconitifolia* plants developed nodules with each of the concentration of *Ascophyllum nodosum* extract applied. The morphology of nodules was recorded and it was observed that *Ascophyllum nodosum* extract treatment led to aggregation of nodules and slight variation in the color. The number of nodules formed was counted upon application of *Ascophyllum nodosum* extract. In both the pot root application as well as pot foliar application, the maximum number of nodules was observed at 0.05% of *Ascophyllum nodosum* extract concentration (Fig. 2 A, B), after which the increase in seaweed concentration was accompanied with decrease in the number of nodules. The study reported that extract of *Ascophyllum nodosum* increased the number of nodules as compared to control which was attributed to better interaction between the root hairs and bacteria present in the seaweed extract. It is well known that Rhizobium and Bradyrhizobium spp., P solubilization, N fixation promote plant growth, and plant productivity. It is also observed from this study that the biofertilizer (ANE) played apositive impact on rhizospheric community and agricultural plant productivity. Hence plant microbes' interaction be redefined as holobionts, an assemblage of different species that forms an ecological unit.

The roots of a plant are known to secrete a battery of molecules to attract favorable microorganisms to form a symbiotic relationship within the rhizosphere (Badri *et al.,* 2009). The application of *A. nodosum* extract increased the number of bacterium, *Sinorhizobium meliloti* in the root nodules of *Medicago sativa* (Khan *et al.,* 2013). Alginates in seaweed extract affect soil properties and promote growth of beneficial microbes. The commercial seaweed extract are known to have no antimicrobial activity against beneficial microbiota of soil and found to enhance the microbial diversity of soil (Alam *et al.,* 2013). Ishii *et al.* (2000) observed that alginate oligosaccharides, produced by enzymatic breakdown of alginic acid mainly extracted from brown algae,

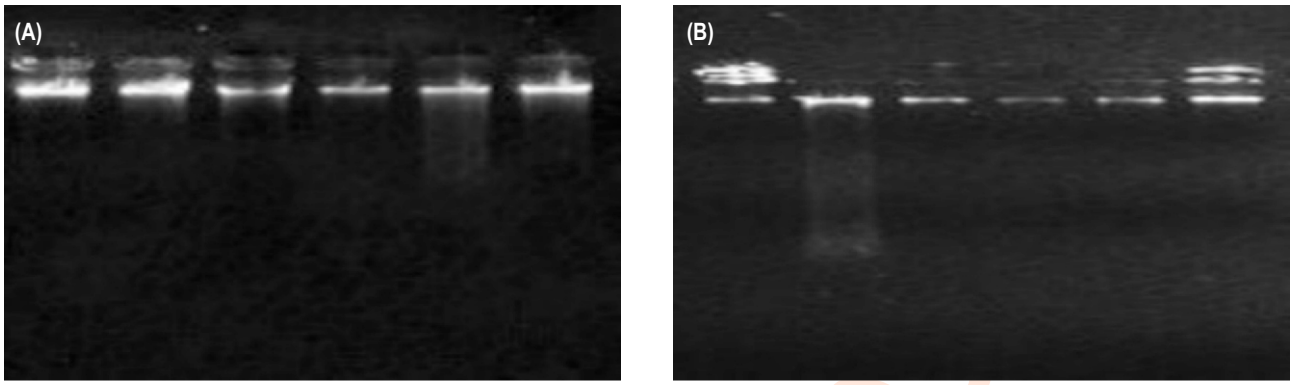


**Fig. 3 :** Quantification of Genomic DNA from (A) Pot foliar application and (B) Pot root application.

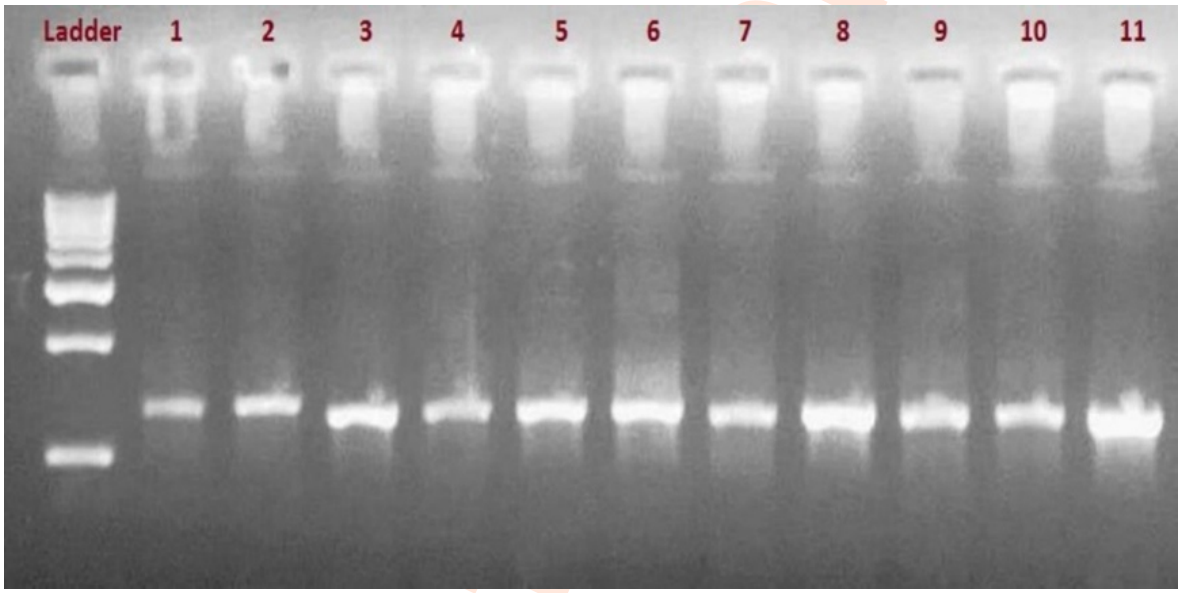


**Fig. 4 :** Amplification of rhizobium isolated from pot foliar and pot root application experiments Ladder-100bp DNA ladder, 1= 0.0 (control), 2= 0.01%, 3= 0.02%, 4= 0.05%, 5= 0.10%, 6= 0.50% *A. nodosum* extract of pot foliar application, 7= 0.01%, 8= 0.02%, 9= 0.05%, 10= 0.10%, 11= 0.50% extract of pot root application.

significantly improves soil microbial community. The seaweed based panchagavya manure on pulses increase nodule development. Better survival and modulating efficiency of rhizobium was observed on application of panchagavya manure (Sangeetha *et al.,* 2010)

Bacterial colonies on YEM agar plates were circular and convex with smooth margins, gummy and translucent. The bacteria were Gram-negative and rod-shaped. The *Rhizobium* cultures from a purified single rhizobial isolates were cultured on fresh YEMA and then inoculated on TY broth for 24 hrs for further molecular studies. The diversity was analyzed using ARDRA. The genomic DNA isolated from the nodulating *Rhizobia* at all the tested concentrations of *Ascophyllum nodosum* extract (Fig. 3 A, B) showed amplification of 16S rDNA gene sequence with forward primer BAC 27F and reverse primer 1378R (Fig. 4). Different restriction pattern was observed when amplified products cut with *Hae II* (Fig. 5) and *Msp I* (Fig. 6) restriction enzymes. The ARDRA and nodule formation data of pot root application coincided well with the concentration of *Ascophyllum nodosum* extract. Dendrogram analysis of rhizobial diversity using ARDRA revealed that the enormous diversity exists among the nodules formed by rhizobia and showed different clusters and sub-clusters in the absence (control) or presence of 0.01, 0.02, 0.05, 0.10 or 0.50% concentration of *Ascophyllum nodosum* extract and the isolates were diversified into two main clusters (Fig. 7). Divergence among isolates of main clusters started at similarity coefficient of 46%. Isolate representing PRA6 formed an out-group in separate cluster whereas other isolates belonged to PRA1, 2, 3, 4 and 5 built first clusters. Further, the first cluster

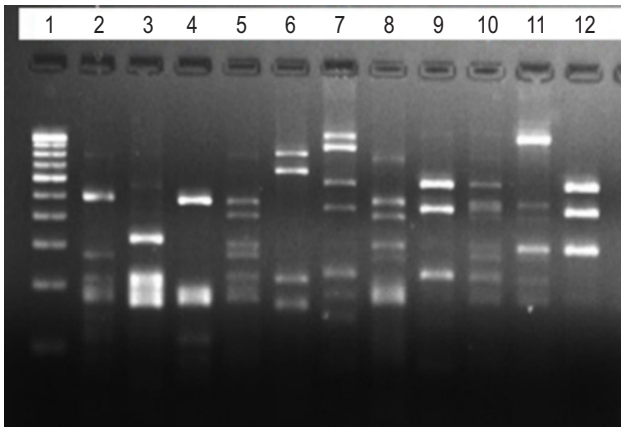


**Fig. 5:** Restriction pattern after cutting with HaeIII in PFA (2-7) and PRA (8-12) experiments. Details of lanes are as: **Lane 1:** 100bp DNA ladder; **Lane 2:** 0.0 (control); **Lane 3:** 0.01% ANE PFA; **Lane 4:** 0.02% ANE PFA; **Lane 5:** 0.05% ANE PFA; **Lane 6:** 0.10% ANE PFA; **Lane 7:** 0.50% ANE PFA; **Lane 8:** 0.01% ANE PRA; **Lane 9:** 0.02% ANE PRA; **Lane 10:** 0.05% ANE PRA; **Lane 11:** 0.10% ANE PRA; **Lane 12:** 0.50% ANE PRA.

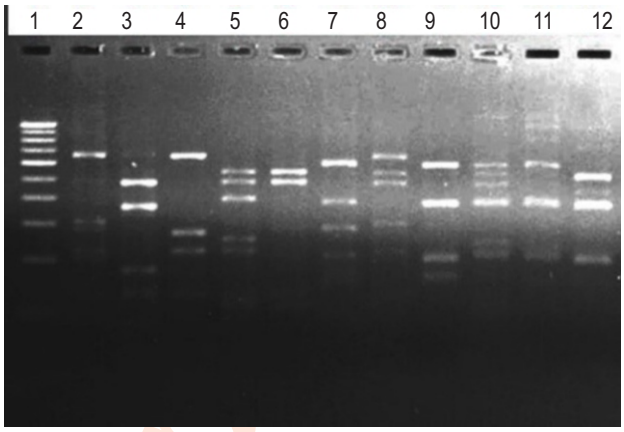


**Fig. 6 :** Restriction pattern after cutting with MSP1 in PFA (2-7) and PRA (8-12) experiments. Details of lanes are as: **Lane 1:** 100bp DNA ladder; **Lane 2:** 0.0 (control); **Lane 3:** 0.01% ANE PFA; **Lane 4:** 0.02% ANE PFA; **Lane 5:** 0.05% ANE PFA; **Lane 6:** 0.10% ANE PFA; **Lane 7:** 0.50% ANE PFA; **Lane 8:** 0.01% ANE PRA; **Lane 9:** 0.02% ANE PRA; **Lane 10:** 0.05% ANE PRA; **Lane 11:** 0.10% ANE PRA; **Lane 12:** 0.50% ANE PRA.

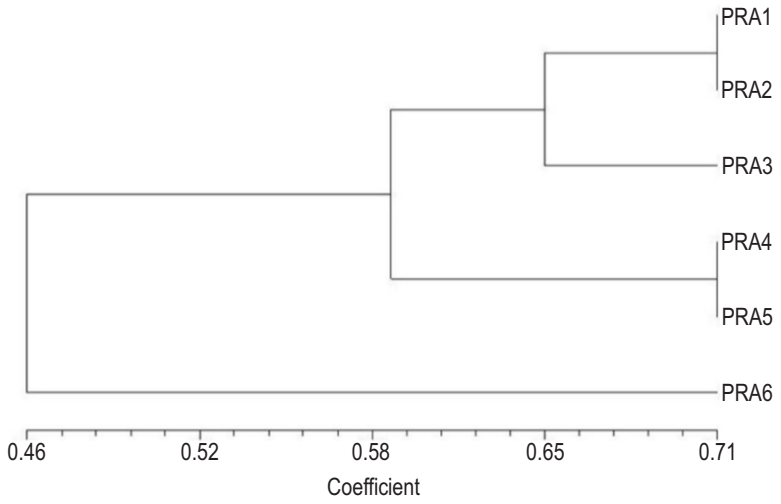


**Fig. 7 :** Denodrogram of rhizobial isolates from nodules of *Vigna aconitifolia* on the basis of double digestion with *HaeIII* and *MspI*.

again diversified into two sub-clusters and divergence among them started approximately at similarity coefficient of 58%. First sub-cluster possessed two isolates of PRA4 and, 5 which were equally similar in 16S rDNA *i.e.* both isolates showed 71% similarity coefficient with each other. Second sub-cluster again diverged into two sub-sub-clusters and the divergence among them started at 65% similarity coefficient. First sub-sub-cluster contained only one isolate representing PRA3 and the second sub-sub-cluster contained two isolates representing PRA1 and 2. The isolates PRA1 and 2 showed similar percentage of 16S rDNA with each other, *i.e.,* 71% similarity coefficient.

The cluster analysis among rhizobial isolates and the effect of seaweed extract on rhizobial nodule diversity can be further explained in different way. Addition of 0.01% of *Ascophyllum nodosum* extract did not alter the rhizobial diversity and thus, the root nodule diversity in control and in the presence of 0.01% of *Ascophyllum nodosum* extract are grouped together in tree (PRA1 and PRA2). The findings of this study corroborates with the reports of Renaut *et al.* (2019), where increased in microbial communities (bacterial and fungal) in tomato and pepper roots was observed on treating with *Ascophyllum nodosum* extract. Organic molecules such as betaines,

polyamines, cytokinins, auxins, oligosaccharides, amino acids and vitamins present in ANE have been found to have overall beneficial productivity effects on plant growth. By surfing the available literature it is now a well-established that biofertilizers can have an impact of the rhizospheric community and agricultural plant productivity. With the increase in the concentration to 0.02% the diversity increased and the branch diverged at coefficient 0.65 to form a second group of PRA3 alone. Further increase in the concentration of sea-weed application to 0.05% increased the diversity and a node was formed at coefficient 0.06. However, further increase in extract concentration to 0.10% did not increase the diversity to form a separate branch and, thus, PRA4 and PRA5 formed third group on the dendrogram. Subsequent increase in the concentration to 0.50% increased the microbial diversity and the fourth group of PRA6 was formed with coefficient 0.46. It may be worth-mentioning that the diversity of PRA6 does not seem to have role in nodule formation, since in the present conditions, the maximum nodule formation was seen in the case of 0.05% *Ascophyllum nodosum* extract, following which the number of nodules per root started declining. ARDRA analysis revealed that resultant microbial diversity due to pot foliar application of extract was not consistent with the concentration of *Ascophyllum nodosum* extract applied (data not shown). The results may be attributed to variation in the effective reach of *Ascophyllum nodosum* extract to roots. All concentrations of ANE applied on foliar region may not reach the roots, the site of action. The experiment was conducted to understand the feasibility of clubbing foliar application in the field along with any other foliar application such as, pesticides for the sake of logistic ease. However, these findings do not support the foliar application unless one is ready to compromise on the wastage of seaweed and efficacy of the spray. To the best of our knowledge, this is the first study to explore the effect of seaweed on microbial diversity of root nodules. Hussain *et al.* (2021) observed that the application of seaweed extract (SWE) improve the growth and root systems, SWE helpful in relatively more exudates into the soil, increasing microbial biomass, hence benefiting the overall soil biogeochemical cycles. More research from the agroecosystem perspective is required to elucidate the collective benefits of using SWE for sustainable agriculture and food production.

The result obtained recommends root application of 0.05% of *Ascophyllum nodosum* seaweed for improving the quantity and quality of nodule formation in *Vigna aconitifolia*. The results suggest future studies to understand the contribution of bioactive components of seaweed on the plant growth parameters, rhizobial diversity and accumulation of important phytoconstituents. *A. nodosum* can natural biofertilzer be used as a beneficial fertilizer to enhance the productivity by enriching the nodulation qualitatively as well as quantitatively in legumes. Further, method used in the present study could be an alternative to culture method to enrich the rhizobial diversity

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**Add-on information**

**Authors' contribution: A.R. Sehrawat:** Design the concept, supervised and wrote the manuscript; **N. Verma:** Carried out the experiments and compile the paper; **K.D. Sehrawat:** Edited Manuscripts and prepared figures; **D. Pandey:** Edited the manuscript.

**Research Content:** The research content is original and has not been published elsewhere

**Ethical Approval:** Not Applicable.

**Conflict of Interest:** The authors declare that they have no conflict of interest.

**Data from other resources:** Not Applicable.

**Consent to publish** : All the authors agree to publish the paper in *Journal of Environmental Biology*.

**References**

Alam, M.Z., G. Braun, J. Norrie and D.M. Hodges: Effect of *Ascophyllum* extract application on plant growth, fruit yield and soil microbial communities of strawberry. *Canad. J. Plant Sci.,* **93**, 23–36 (2013).

Allen, O.N. and E.K. Allen: The Leguminosae. A Source Book of Characteristics, Uses and Nodulation. 1st Edn., University of Wisconsin Press, Madison, Wisconsin, USA. pp. 812 (1981).

Arun, D., P. K. Gayathri, M. Chandran and D. Yuvaraj: Studies on effect of seaweed extracts on crop plants and microbes. *Int. J. Chem. Tech. Res.,* **6**, 4235–40 (2014).

Ausubel, F., R. Brent, R.E. Kingston, D.D. Moore, J.G. Seidman, J.A. Smith and K. Struhl: Short protocols in Molecular Biology. 5th Edn., 2 Volume set. John Wiley Sons, Inc., New York, USA. pp. 1504 (2002).

Badri, D. V., T. L.Weir, D. V. D. Lelie and J. M. Vivanco: Rhizosphere chemical dialogues: Plant–microbe interactions. *Curr. Opini. Biotechnol.*, **20**, 642–650 (2009).

Bringer, J.E.: R factor transfer in *Rhizobium leguminosarum*. *J. Gen. Microbiol.,* **84**, 188-198 (1974).

Brockwell, J., P.J. Bottomley and J.E. Thies: Manipulation of rhizobia microflora for improving legume productivity and soil fertility: A critical assessment. *Plant Soil.,* **174**, 143–180 (1995).

Chunekar, K.C. and G.S. Pandey: Bhavaprakash Nighantu (Indian Materia Medica) of Sri Bhavamisra (c. 1500–1600 AD). Chaukhamba Bharati Academy, Uttar Pradesh, India. 984 pages (1998).

Dhargalkar, V.K. and N. Pereira: Seaweed: Promising plant of the

millennium. *Sci. Cult.,* **71**, 60-66 (2005).

Fred, E.B., I.L. Baldwin and E. McCoy: Root nodule bacteria and leguminous plants. 5th Edn., Libraries Parallel Press., Madison, Wisconsin, USA. 343 pages (1932).

Fujihara, S., J. Terakado, M. Takenaka and T. Yoneyama: Specific occurrence of phenethylamine in root nodules formed from *Bradyrhizobium*-legume symbiosis. *Plant Soil,* **238**, 123-132 (2002).

Hussain, H.I., N. Kasinadhuni and T. Arioli: The effect of seaweed extract on tomato plant growth, productivity and soil. *J. Appl. Phycol.*, **33**, 1305 -1314 (2021).

Ishii, T., J. Aikawa, S. Kirino, H. Kitabayashi, I. Matsumoto and K. Kadoya: Effects of alginate oligosaccharide and polyamines on hyphal growth of vesicular-arbuscular mycorrhizal fungi and their infectivity of citrus roots. In: Proceedings of 9th International Society of Citri culture Congress, *Orlando,* pp. 1030–1032 (2000).

Choi, S.K., H.J. Bae, S.J. Kim and I.S. Choi: *In-vitro* antibacterial and anti-inflammatory properties of seaweed extracts against acne inducing bacteria, *Propionibacterium acnes J. Environ. Biol.,* **32**, 313-318 (2011).

Khan, W., R. Zhai A, Souleimanov , A.T. Critchley, D.L. Smith and B. Prithiviraj: Commercial extract of *Ascophyllum nodosum* improves root colonization of alfalfa by its bacterial symbiont *Sinorhizobium meliloti*. *Comm. Soil Sci. Plant Ana.,* **43**,18 (2012).

Khan, W., R. Palanisamy, A.T. Critchley and D.L. Smith, Y. Papadopoulos and B. Prithiviraj: *Ascophyllum nodosum* extract and its organic fractions stimulate *Rhizobium* root nodulation and growth of *Medicago sativa* (Alfalfa). *Communic. Soil Sci. Plant Analy.,* **44**, 900–908 (2013).

Long, S.R.: *Rhizobium* symbiosis: Nod factors in perspective. *Plant Cell,* **8**, 1885– 1898 (1996).

Lukow, T., P.F. Dunfield and W. Liesack: Use of the T-RFLP technique to assess spatial and temporal changes in the bacterial community structure within an agricultural soil planted with transgenic and non-transgenic potato plants. *FEMS Microbiol. Ecol.,* **32**, 241–247 (2000).

Renaut, S., J. Masse, J.P. Norrie, B. Blal and M. Hijri: A commercial seaweed extract structured microbial communities associated with tomato and pepper roots and significantly increased crop yield. *Microbial. Biotechnol.,* **12**, 1346–1358 (2019).

Rohlf, F.J.: NTSYS PC Numerical Taxonomy and Multivariate Analysis System Version 2.0 User Guide. Applied Biostatistics Inc., Setauket, New York, 37 pages (1998).

Rungruangmaitree, R. and W. Jiraungkoorskul: Pea, *Pisum sativum* and its anticancer activity. *Pharmacogn. Rev.,* **11**, 39–42 (2017).

Sangeetha, V. and R. Thevanathan: Biofertilizer potential of traditional and panchgavya amended with seaweed extract. *J. American Sci.,* **6**, 61-67 (2010).

Sathya, B., H. Indu, R. Seenivasan and S. Geetha: Influence of seaweed liquid fertilizer on the growth and biochemical composition of legume crop, *Cajanus cajan* (L). Mill sp. *J. Phytol.,* **2**, 50–63 (2010).

Somasegaran, P. and H.J. Hoben: Handbook for *Rhizobia*. Methods in Legume-Rhizobium Technology. 1st Edn. Springer Verlag, New York (1994).

Sylvie, B. and A.N. Patrick: *Phaseolus vulgaris* response to *Rhizobium* inoculation, lime and molybdenum in selected low pH soil in Western Cape, South Africa. *African J. Agricul. Res.,* **5**,1804-1811 (2010).

Ugarte, R.A., J.S. Craigie and A.T. Critchley: Fucoid flora of the rocky intertidal of the Canadian Maritimes: Implications for the future with rapid climate change. In: Seaweeds and their roles in globally changing environments (Eds.: A. Israel, R. Einav and J. Seckbach). Springer, New York, pp.69–90 (2010).

Velazquez, E., P. Garcia-Fraile, M.H. Ramirez-Bahena, R. Rivas and E. Martinez-Molina: Bacteria involved in nitrogen-fixing legume symbiosis: Current Taxonomic Perspective in Microbes for Legume Improvement (Eds.: M.S. Khan, A. Zaidi and J. Musarrat). Springer, Vienna. pp. 1-25 (2010).

Verma, N., A.R. Sehrawat, D. Pandey and B.K. Pandey: Seaweed: A novel organic biomaterial. *Curr. J. Appli. Sci. Technol.,* **39**, 1–8 (2020).

Verma, N., K.D. Sehrawat, S. Kumari and A.R. Sehrawat: Antityrosinase activity and photosynthetic pigments in seaweed treated sprouts of *Vigna acontifolia*. *Indian J. Agric. Sci.,* **89**, 57-59 (2019).

Verma, N., K.D. Sehrawat, A. Ahlawat and A.R. Sehrawat: Legumes: The natural products for industrial and medicinal importance-A review. *Int. J. Cell Sci. Biotechnol.,* **6**, 6-13 (2017).

Verma, N., K.D. Sehrawat and A.R. Sehrawat: Studies on the effect of *Ascophyllum nodosum* extract (ANE) on growth, antioxidant potential and alpha glucosidase inhibition activity of *Vigna aconitifolia* (RMO 225). *Int. J. Curr. Microbiol. Appli. Sci.,* **6**, 2125–2138 (2017).

Vietmeyer N.D.: Lesser-known plants of potential use in agriculture and forestry. *Science*, **232**, 1379-84 (1986).

Watt, G., E. Thurston and T. Mukhopadhyaya: A Dictionary of Economic Products of India. 1st Edn. Cosmo Publications, Delhi, India (1889).

Wu, T.Y. and C.H. Lin: Analysis of cytokinin activity in commercial aqueous extract. *Gar. Buwi. Sch.,* **65**,170-173 (2000).

Zhai, R.: Effects of brown seaweed, *Ascophyllum nodosum*, on the nodulation and growth of Alfalfa. Dissertation Dalhousie University Halifax, Nova Scotia (2012).