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Bio-Inoculants and Vermicompost Influence On Yield, Quality of *Andrographisalata*, and Soil Properties

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Abstract
*Andrographisalata*is a source of diterpenoids and 2 -oxygenated flavonoids, which are of utility in pharmaceutical industry and ayurvedic formulations. With the aim of producing quality herb, an experiment was conducted with different combinations of bio-inoculants and vermicompost (VC) in controlled condition. It was observed that the highest Leaf: Stem (L: S) ratio, fresh herb yield and andrographolide yield was recorded when the soil was incorporated with VC along with *Azotobacterchrococcum.* Further there was a significant improvement in all soil fertility parameters. However, when all the bio-inoculants (*Azotobacterchrococcum + Azospirillumbrasilesence*) were mixed with VC, there was a significant improvement in soil dehydrogenase, alkaline and acidic phosphatase activity. A positive correlation coefficient could be derived amongst plant growth, yield and soil properties. The study suggests that application of the bio-inoculants and organic fertilizers can enhance productivity while maintaining the desired quality of the herb.

Keywords
Andrographisalata, andrographolide, bio-inoculants, organic fertilizers, soil fertility, vermicompost.

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INTRODUCTION
Andrographis is a plant that is native to South Asian countries such as India and Sri Lanka. The leaf and underground stem are used to make medicine. Andrographis is frequently used for preventing and treating the common cold and flu (influenza). Some people claim Andrographis stopped the 1919 flu epidemic in India, although this has not been proven (Karmegam*et al.,* 2015).

Andrographis is also used for a wide assortment of other conditions. It is used for digestive complaints including diarrhoea, constipation, intestinal gas, colic, and pain; Andrographis is also used as astringent, bacteria killing agent, painkiller, fever reducer, and treatment for worms (Karmegam*et al.,* 2015).

Bio fertilizer is a substance which contains living or latent microorganisms which, when applied to seed, plant root or soil, colonizes the rhizosphere or the interior of the plant and promotes growth by providing

and essential nutrients or increasing the supply or availability of primary nutrients to the host plant (Alam, S., Khalil, S., Ayub, N. and Rashid, M. 2002).

Vermicompost is produced by biodegradation of organic material through interactions between earthworms and micro-organisms. There is the presence of nutrients such as nitrates, phosphates and exchangeable calcium and soluble potassium in vermicompost. Vermicompost contains plant growth influencing materials produced by microorganisms (Donahue and Murphy, 1981).

Azotobacter sp. is an obligate aerobe, although it can grow under low O_2 concentration. The ecological distribution of this bacterium is a complicated subject and is related with diverse factors which determine the presence or absence of this organism in a specific soil (Alam, S., Khalil, S., Ayub, N. and Rashid, M. 2002).

Azospirillumsp are generally regarded as rhizosphere bacteria, but display strain-specific deference's in the way they colonize roots. *Azospirillum* can convert atmospheric nitrogen into ammonium under micro aerobic conditions at low nitrogen levels, through the action of the nitrogenase complex. This enzyme is built from two components: the dinitrogenase protein (MoFe protein, NifDK), which contains a molybdenum-iron cofactor, is the site of N2 reduction. The dinitrogenase reductase protein (Fe protein, NifH) transfers electrons from an electron donor to the nitrogenase protein (Okon, Y. and Vanderleyden, J. 1997).

MATERIALS AND METHODS

Study site

The study was conducted at Hindusthan College of Arts and Science College, Coimbatore, Tamil Nadu, India.

Collection of soil sample

The soil samples were collected at 0.30 cm soil depth (Dickman*et al.,* 1984) from garden area of Hindusthan College of Arts and Science College, Coimbatore, Tamil Nadu, India. Collected samples were mixed and placed in sterile container and then brought to the laboratory.

Isolation of the organisms

Azotobacter: For the isolation of Azotobacter, the soil slurry was made by suspending 1 g of the collected dry soil in 100 ml nitrogen free mannitol broth. Incubate the broth at 30°c for 4-6days, the growth was observed as thin floating layer upon the broth. Then the layer was taken using sterile inoculation loop and streaked on the Azotobacter isolation agar, incubated at 30°C for 2-4days.

Azospirillum:

For the isolation of Azospirillum, the soil slurry was made by suspending 1 g of the collected dry soil in 100 ml nitrogen free malate broth. Incubate the broth at 30°c for 4-6days, the growth was observed as thin floating layer upon the broth. Then the layer was taken using sterile inoculation loop and streaked on the Azotobacter isolation agar, incubated at 30°C for 2-4days.

Vermicompost

5 kg of vermicompost was collected from Tamilnadu Agricultural University, Coimbatore.

Preparation of the inoculum

The inoculum was prepared by inoculating the culture into one-liter nitrogen free mannitol broth and nitrogen free malate broth. Then the broth is incubated. After incubation the inoculums was used for seedling treatment (Brick *et al.,* 1991).

Seedling treatment

One month old seedlings were procured from the seed technology division of IFGTB. The seedlings were maintained under nursery conditions. The seedlings were treated with 10 ml of Azotobacter and Azosprillum for 12 weeks and the growth parameters were observed (Das *et al.,* 2013).

Crop cultivation

Experiment was carried out in cylindrical pots to avoid the exchange of microbial strains and soil nutrients among the treatments for the evaluation of bio-inoculants efficiency. *Andrographisalata* was taken for this study. It was planted on five pots with different bio inoculants

- control (untreated soil)
- vermicompost (VC)
- vermicompost + *Azotobacterchrococcum*
- vermicompost + *Azospirillumbrasilesence*
- vermicompost + all three bio inoculants (vermicompost + *Azotobacterchrococcum +Azospirillumbrasilesence*)

Growth parameters

The growth parameters such as shoot length, root length and diameter of the shoot were measured after 12 weeks of treatment with bio inoculants to the seedlings. The shoot diameter is measured using a Vernier caliper.

Weighing the wet and dry weight of the plant

Measuring wet weight: The plants were removed from the soil and the soil was washed off. Then the plants were blotted with soft paper to remove the surface moisture. Then the plants were weighed.

Measuring dry weight: The plants were removed from the soil and the soil was washed off. Then the plants were blotted with soft paper to remove the surface moisture. The plants were dried in an oven at a low heat of 100°F for overnight. Then the plants were weighed.

Root mass and shoot mass

The plants were removed from the soil and the soil is washed off. Then the roots and shoots were cut down and are separately weighed.

Soil analysis-chemical and biological analysis

Initial and post-harvest was done

Soil pH

Take 20g soil in 100 ml beaker and add 40 ml of distilled water to it. The suspension is stirred at a regular interval for 30 minutes determine the pH by immersing electrodes in suspension.

Soil organic carbon

Walky black method was done to examine soil organic carbon.

Available phosphorus

Determination of available phosphorous was done by Olsen method.

Soil potassium:

1. Scoop 2 g of prepared soil into an extraction flask. (See Chapter 2 for scooping techniques. Use the appropriate number of blanks and reference samples per laboratory quality assurance/quality control procedures.)
2. Add 20 mL of extracting solution to the extraction flask. (Note: The quantity of soil and extracting solution may be varied as long as the 1:10 ratio is maintained.)
3. Shake for 5 minutes on the shaker at 200 rpm. Recheck speed weekly.
4. Filter the suspensions through Whatman No. 2 or equivalent filter paper. Refilter or repeat if the extract is cloudy.
5. Set up the atomic adsorption/emission spectrometer for K by emission. After warmup, determine the standard curve using the standards and obtain the concentrations of K in the soil extracts.
6. To convert K concentration (ppm) in the soil extract solution to ppm in a soil (mg K/kg), multiply by 10. To convert to pounds of K per acre, multiply by 20.

Soil Exchangeable Ca, Mg and Na

Reagents

1. Extracting Solution: 1 M NH_4OAc at pH 7.0. Mehlich 3 may be substituted for non-calcareous soils.
2. Standards Using a protocol similar to that for K, make up a 1,000 ppm stock solution of Ca ($CaCO_3$ dissolved in a minimum of HCl), Mg (Mg metal dissolved in HCl) and Na (NaCl). Commercially available stock solutions may be used. Make working standards for Ca of 0, 10, 20, 30, 40 and 50 ppm and Mg of 0, 1, 2, 3, 4 and 5 ppm using the extracting solution and sufficient lanthanum to give a final concentration of 1percent (wt./vol.). Concentrations of the Na working standards should be the same as for K; 0, 10, 20, 30, 40 and 50 ppm.

Procedure:

Follow the procedure as outlined for K. The same extracts used for K may be used for Ca, Mg and Na. Dilution will be necessary. Make dilutions for Ca and Mg analysis with the extracting solution containing 1 percent lanthanum (wt. /vol.). The final dilution for Ca and Mg analysis should contain the same lanthanum concentration as the working standards. Ca and Mg are determined by atomic adsorption and Na by emission. The results will be expressed in the same units as for K.

Biological analysis

Soil microbial load

Azotobacter: 1 gm of soil sample was taken and mixed in 100 ml of sterile distilled water and then serially diluted upto 10^2 to 10^8. Azototobacter isolation agar medium was plated and the serially diluted sample was spread over the plates and then incubated at 30°Cfor 24 to 48 hours.

Azospirillum: 1 gm of soil sample was taken and mixed in 100 ml of sterile distilled water and then serially diluted up to 10^2 to 10^8. Azospirillum agar medium was plated and the serially diluted sample was spread over the plates and then incubated at 30°Cfor 24 to 48 hours.

Then the colonies were counted using colony counter, the CFU/ml can be calculated using the formula:

Cfu/ml = (no. of colonies x dilution factor) / volume of culture plate

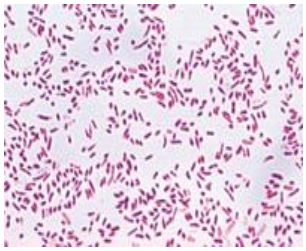
Harvesting and HPLC analysis

Above ground biomass of *A.alata* was harvested (after the 12 weeks) at the flowering initiation as the active compound andrographolide content is optimum at this stage. Since the whole plant contains active compound, entire harvested material was shade dried and stored. Plant material was dried at 45 ◦ C for 72 h and pulverized to powder. 100 mg of pulverized leaves was extracted three times with 10 ml methanol on water bath for 30 min. All three filtrates into one with total volume 30 ml and evaporated to dryness. The final volume was made up to 1 ml and passed through 0.45 m filter. 20 l of this extract was injected into reverse-phase HPLC system. All analyses were conducted with waters HPLC system consisting of waters 717 plus auto sampler, waters 600 pump; in line solvent degasser, Empower 22 software and 2296 photodiode array detector. The separation was carried out using a waters (X Bridge) C18 column (250 × 4.6, 5 m). Guard column had 4.6 × 20 mm guard cartridge. The mobile phase consisted of 70% water and 30% acetonitrile with isocratic run and the flow rate was 0.8 ml/min. UV detection was done at 230 nm. The andrographolide content was determined using external standard of andrographolide,

RESULTS

The colonies of Azotobacter was analyzed using staining and plating technique Colonies were isolated in Azotobacter isolation agar medium and gram negative rod colonies were observed.

Figure 1. Azotobacter on azotobacter isolation agar



igure.2 Microscopic observations of Azotobacter isolate

The colonies of Azospirillum was analysed using staining and plating technique. Colonies were isolated in Azospirillum medium and gram negative colonies were observed.

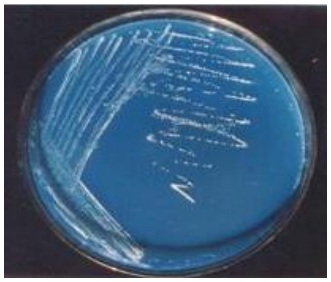


Figure. 3Azospirillum on Azospirillum medium

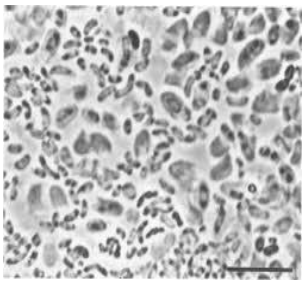


Figure. 4Microscopic observation of Azospirillum isolate

The vermicompost was prepared using *Eiseniafetida* (earthworm species).

Figure. 5 vermicompost preparation

The seedling process was done by mass cultivation of microbial inoculum (*Azotobacter sp.* and *Azospirillum sp.*) and *Andrographisalata*seedling inoculation.

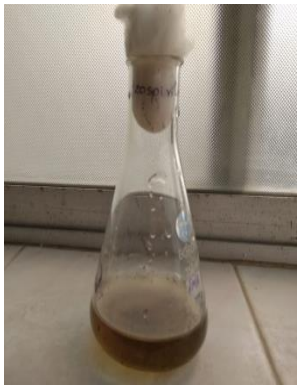


Figure. 6 Inoculum preparation

seedling process

Figure.7Azospirillum inoculation

Figure. 8Azotobacterinoculation

Seedlings of *Andrographisalata*

Figure. 9 Inoculated seedlings in pots

Soil pH

Table 1. Soil pH was identified for all treatments

S.no	Treatments	Soil pH
1	T1	8.9
2	T2	8.0
3	T3	9.1
4	T4	8.4
5	C	9.3

- T1 - Treatment 1(Vermicompost)
- T2 - Treatment 2(Vermicompost +*Azotobacter)*
- T3 - Treatment 3(Vermicoompost+*Azospirillum)*
- T4 - Treatment 4(Vermicompost+*Azotobacter+Azospirillum)*
- C - Control

Measurements before treatment

Table 2. Number of leaves in plants before treatment

T4 plant showed the best result when compared to other treated plants

S.no	Treatment	No.of leaves
1	T1	5
2	T2	4
3	T3	6
4	T4	7
5	C	5

- T1 - Treatment 1(Vermicompost)
- T2 - Treatment 2(Vermicompost +*Azotobacter)*
- T3 - Treatment 3(Vermicoompost+*Azospirillum)*
- T4 - Treatment 4(Vermicompost+*Azotobacter+Azospirillum)*
- C - Control

Table 3. Surface area of the leaves:

T3 plant showed the best result when compared to other treated plants

S.no	Treatment	Maximum leaf surface area (cm^2)	Minimum leaf surface area(cm^2)
1	T1	7.2	4.5
2	T2	6.5	3.9
3	T3	7.8	3.6
4	T4	6.9	5.3
5	C	7.0	4.2

- T1 - Treatment 1(Vermicompost)
- T2 - Treatment 2(Vermicompost +*Azotobacter)*
- T3 - Treatment 3(Vermicoompost+*Azospirillum)*
- T4 - Treatment 4(Vermicompost+*Azotobacter+Azospirillum)*
- C - Control

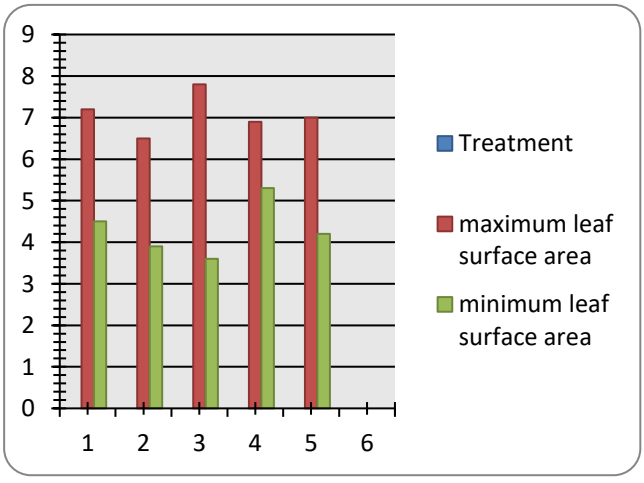


Figure. 10 Surface area of leaves

Table 4. Total number of leaves:

T3 plant showed the best result when compared to other treated plants

S.no	Treatment	No of leaves (untreated)	No of leaves(treated)
1	T1	27	92
2	T2	32	40
3	T3	30	95
4	T4	35	103
5	C	33	47

- T1 - Treatment 1(Vermicompost)
- T2 - Treatment 2(Vermicompost +*Azotobacter)*
- T3 - Treatment 3(Vermicoompost+*Azospirillum)*
- T4 - Treatment 4(Vermicompost+*Azotobacter+Azospirillum)*
- C - Control

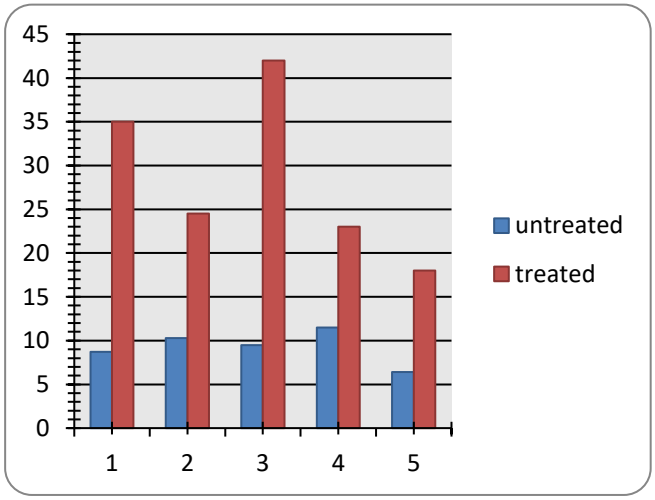


Figure. 11 Total number of leaves

Table 5. Total number of branches:

T1 plant showed the best result when compared to other treated plants

S.no	Treatment	No of branches (untreated)	No of branches (treated)
1	T1	4	17
2	T2	2	4
3	T3	5	12
4	T4	4	10
5	C	3	4

- T1 - Treatment 1(Vermicompost)
- T2 - Treatment 2(Vermicompost +*Azotobacter)*
- T3 - Treatment 3(Vermicoompost+*Azospirillum)*
- T4 - Treatment 4(Vermicompost+*Azotobacter+Azospirillum)*
- C - Control

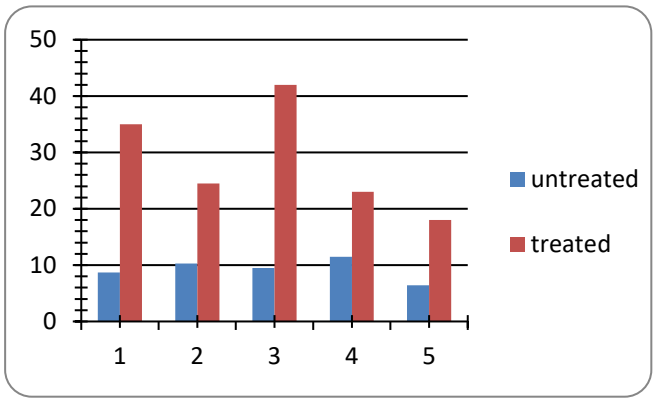


Figure. 12 Total number of branches

*Andrographisalata*plant is harvested and taken for measurement of shoot and root

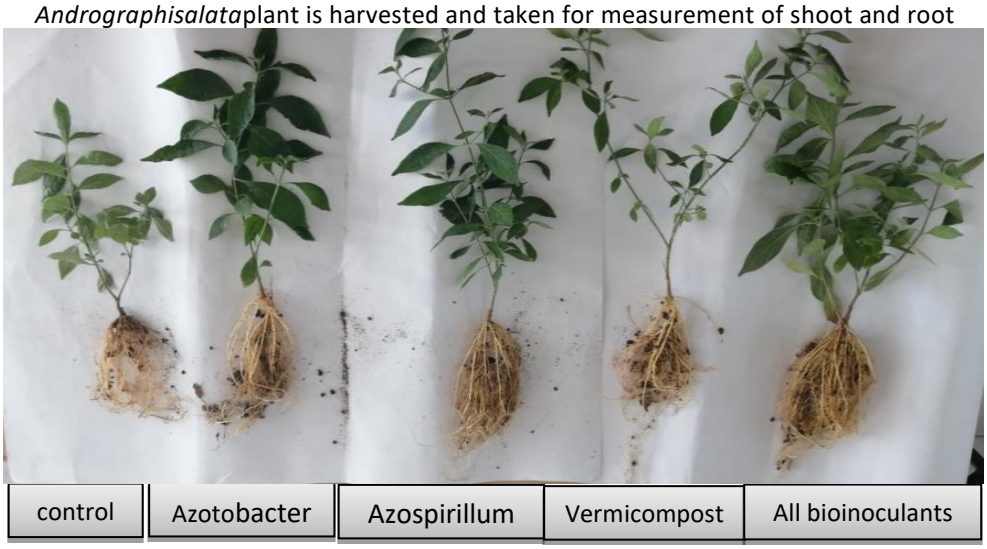


Figure. 13 Seedlings after measurement

Measurements after treatment

Table 6. Length of the root, shoot and the total length of the seedling

T1 plant showed the best result when compared to other treated plants

S.no	Treatment	Treated			Untreated		
		Total length(cm)	Root length	Shoot length	Total length(cm)	Root length	Shoot length
1	T1	66	31	35	15.9	7.2	8.7
2	T2	48.5	24	24.5	19.8	9.5	10.3
3	T3	69	27	42	17.3	7.8	9.5
4	T4	55	32	23	24.5	13	11.5
5	C	44	25	18	14.7	8.3	6.4

- T1 - Treatment 1(Vermicompost)
- T2 - Treatment 2(Vermicompost +*Azotobacter)*
- T3 - Treatment 3(Vermicoompost+*Azospirillum)*
- T4 - Treatment 4(Vermicompost+*Azotobacter+Azospirillum)*
- C - Control

Table 7. Total weight of the plants

T4 plant showed the best result when compared to other treated plants

S.no	Treatment	Total weight of the plants in gm(untreated)	Total weight of the plants in gm(treated)
1	T1	5.2	13.6
2	T2	6.1	13.4
3	T3	5.4	19
4	T4	4.9	24.2
5	C	5.1	9.3

- T1 - Treatment 1(Vermicompost)
- T2 - Treatment 2(Vermicompost +*Azotobacter)*
- T3 - Treatment 3(Vermicoompost+*Azospirillum)*
- T4 - Treatment 4(Vermicompost+*Azotobacter+Azospirillum)*
- C - Control

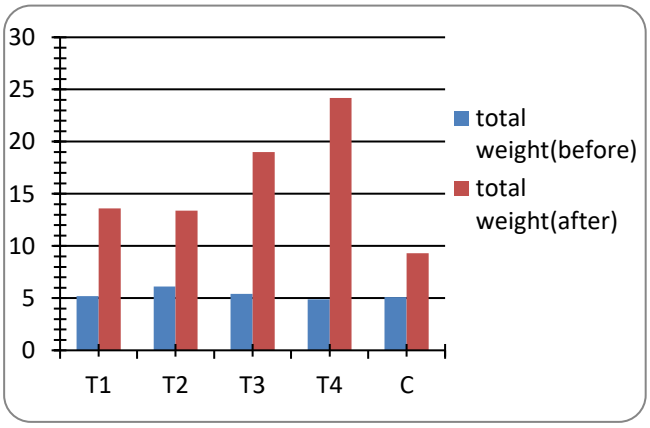


Figure. 14 Total weight of the plants

Andrographisalata harvesting and extract preparation for HPLC analysis

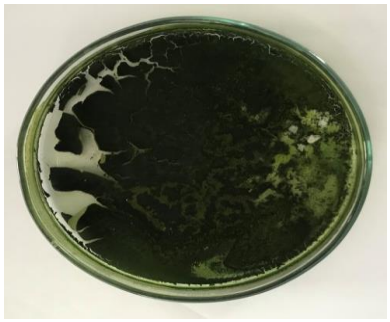


Figure. 15 *Andrographisalata* (methanol) extract

Soil tests

Table 8. Soil test results

Parameter	Value	Unit	Comments
pH	8.40	-	Slightly alkaline
Available nitrogen	146	kg ha^{-1}	Low
Available phosphorous	14.0	kg ha^{-1}	Medium
Available potassium	511	kg ha^{-1}	High

Microbial load of *Azotobacter*

The colony forming unit of *Azotobacter* was calculated by the formula
Cfu/ml = (no. of colonies x dilution factor) / volume of culture plate

Table 9. Colony forming unit per ml for *Azotobacter*

s.no	Dilution factor	No of colonies	Cfu per ml
1	10^{-1}	102	102×10^{2}
2	10^{-2}	98	98×10^{3}
3	10^{-3}	84	84×10^{4}
4	10^{-4}	69	69×10^{5}
5	10^{-5}	54	54×10^{6}
6	10^{-6}	49	49×10^{7}
7	10^{-7}	30	30×10^{8}

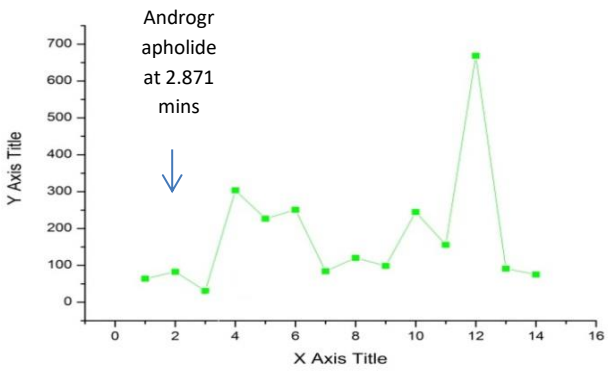
The colony forming unit of *Azospirillum* was calculated by the formula
Cfu/ml = (no. of colonies x dilution factor) / volume of culture plate

Table 10. Colony forming unit per ml for *Azospirillum*

s.no	Dilution factor	No of colonies	Cfu per ml
1	10^{-1}	94	94×10^{3}
2	10^{-2}	83	83×10^{4}
3	10^{-3}	76	76×10^{5}
4	10^{-4}	68	68×10^{6}
5	10^{-5}	46	46×10^{7}
6	10^{-6}	28	28×10^{8}
7	10^{-7}	19	19×10^{9}

HPLC Analysis

In HPLC analysis the andrographolide component in vermicompost inoculated*Andrographisalata*plant is higher than the *Azotobacter and Azospirillum inoculated Andrographisalata*plant. The standard Andrographolide took 2.871 minutes as retention time



The peak indicates that the andrographolide content in the vermicompost treated *Andrographisalata*plant, harvested after 90 days of plantation i.e. just before flowering making it ideal harvesting time. The andrographolide content also depends on growing region and season.

DISCUSSION

Azotobacter and *Azospirillum*are well known group of bacteria which is symbiotically associated with leguminous plants and act as primary nitrogen fixer of nitrogen. These strains were isolated from soil.

The use of phosphate solubilising bacteria as bio-inoculants increases the P uptake by plants (Igual*st al.,* 2001).

To improve the growth of the plant *Andrographisalata,* bio-fertilizers were applied to enhance its growth. Since the plant *Andrographisalata*has many medicinal values and it's being enlarged, the study is carried out to improve its growth by applying bio-fertilizers. These bio-fertilizers are the beneficial microbe that protects the soil fertility and enhance the plant growth whereas chemical fertilizers deplete the soil fertility and fertility and harm the plant growth.

Use of fungicide would result in accumulation of toxic compounds which is more hazardous to human population and the environment. In order to overcome these issues, bio-control by an antagonism method is being employed.

Vermicompost is the good organic bio-fertilizer to increase the yield of the andrographolide content in *Andrographisalata*plant when compared to other bio-inoculants

SUMMARY AND CONCLUSION

The study concludes that *Andrographisalata* responds firmly to different applications of organic amendments strengthening the fact that organic production technology can be adopted for medicinal plants which further fetches value in ayurvedic or traditional medicines, while harmonising natural resource and nature.

The pot culture method was carried out in this process, bio-inoculants and vermicompost are inoculated in separate pots and the bio inoculants are inoculated into *A.alata*by seedling treatment method and allowed to grow for harvesting was done after 90 days. Then the methanol extract was prepared, more number of components are seen in the extract, HPLC analysis was done at 230nm for separation of andrographolide compound.

The andrographolide compound was increased by microorganisms present in the soil they can be varied for different bio-inoculants; the soil fertility was differed for each pots because of the microorganisms. Available micro and macronutrients (carbon, nitrogen, Sulphur, phosphorous, potassium) are estimated using different methods.

Hence the andrographolide component is increased in vermicompost inoculated in *A.alata.*Andrographolide component has the pharmacological activity such as Anti-inflamatory, hepatoprotective, antioxidant, antipyretic, anthelmintic, antiviral, anti-hepatitis C virus activity, antihyperglycemic activities these are more useful for living beings. Andrographolide in *A.alata*has antibacterial and antivenom activity.

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