Educational Administration: Theory and Practice
2024, 30(1), 1680-1687
ISSN: 2148-2403
https://kuey.net/

Research Article

Effect Of Different Consortia Of Bioinoculants On The Growth And Nutrient Content Of Andrographis Paniculata

Dr Shubha[1], Prathibha K Y[2*], Manjula A C[3]

[1]Professor, Department of Botany, Government First Grade College, Vijayanagara, Bengaluru 560104
[2*]Professor, Department of Botany, Maharani Cluster University, Palace Road, Bengaluru, Karnataka, India, 560001
[3]Professor, Department of Sericulture, Maharani Cluster University, Palace Road, Bengaluru, Karnataka, India, 560001

***Corresponding author:** Prathibha K Y
*Email: kyprathibha3@gmail.com

Citation: Prathibha K Y, et.al (2024) Effect Of Different Consortia Of Bioinoculants On The Growth And Nutrient Content Of Andrographis Paniculata, *Educational Administration: Theory and Practice, 30(1), 1680-1687*
Doi: 10.53555/kuey.v30i1.6524

ABSTRACT

Andrographis paniculata is an endangered member of the family Acanthaceae. The plant is used in many types of herbal medicines for liver disorders and also found to have anti-HIV properties. Various combinations of bioinoculants were used to evaluate the efficacy of the bioinoculants on the growth, biomass yield, nutrient content and mineral element content of the plant. The bioinoculants used were Glomus mosse, Glomus fasiculatum, Azotobacter, Aspergillus awamori, Frateuria aurentia and biocotrol agent Trichoderma viride. Combined inoculation of Glomus fasiculatum, Azotobacter, Aspergillus awamori, Frateuria aurentia and Trichoderma viride resulted in maximum biomass yield compared to control and other combinations whereas maximum root length was achieved in Frateuria aurentia alone treated plants. More than two fold increase in NPK concentration was observed in the plants treated with the above mentioned combination of bioinoculants. Mineral elements viz., Calcium and Magnesium content was highest in the combined inoculation whereas no change was observed in the Sodium content which was same in all the plants

Keywords: Bioinoculants, Andrographis paniculate , Glomus, Azotobacter, Aspergillus awamori, Frateuria aurentia ,Trichoderma viride

Results: Cent percent germination was observed in seeds treated with 1N HCl for 10min and 0.1%GA3 for 20min whereas 1N HCl (10min) and 0.1% IBA(20 min) showed 95% germination. Viability of seedlings was maximum in 1N HCl and 0.1% IBA treated seedlings. Combined inoculation with T2- Glomus fasciculatum + Azotobacter +Aspergillus awamori + Frateuria aurentia + Trichoderma viridae showed significant increase in plant height of kalmegh followed by T5 that is Frateuria aurentia and T1-Glomus mosse + Azotobacter +Aspergillus awamori +Fraturia aurentiar +Trichoderma viridae compared to single inoculation and uninoculated control.

Conclusion: As the plant is critically endangered and is in great demand a consortia of microbes viz., Glomus fasciculatum + Azotobacter +Aspergillus awamori + K solubiliser +Trichoderma viridae can be effectively used to get better growth and yield of Andrographis paniculata and thereby reducing the exploitation of the plant from the wild and conserving them in their natural habitat.

Keywords: Andrographis paniculata , Glomus mosse , Glomus asiculatum, Azotobacter, Aspergillus awamori, Frateuria aurentia ,Trichoderma viride, bioinoculants.

INTRODUCTION

https://www.hpcismart.com/images/website/ManChemNews/DIR_193/F_99622.jpg

Medicinal plant is an integral part of human life to combat the sufferings from the dawn of civilization . It is estimated that more than 80,000 of total plant species have been identified and used as medicinal plants around the world .The popularity of herbs among health consumers as an alternative medicine to alleviate diseases and health maintenance are due to its reputation of being safer and cheaper than synthetic medicines. Herbs are also easily available through cultivation practice, either in mass propagation or smaller scale planting **[1].** One of the more popular herbs among the Asian population is *Andrographis paniculata* , also known as the 'king of bitters' and much used in Ayurvedic medicine. It belongs to the family Acanthaceae. It is extensively used in Indian systems of medicine like Ayurveda, Siddha and Unani. The plant is used as tonic ,blood purifier, antihelmenthic, antipyretric, anti-inflammatory and for snakebite, dog bite, constipation, skin disease. It has antityphoid, antibiotic properties and also anti-HIV potential **[2].** AP is used as a traditional herbal medicine in Bangladesh, China, Hong Kong, India, Pakistan, Philippines, Malaysia, Indonesia, and Thailand **[3].** The aerial part of AP is most commonly used, its extracts contain diterpenoids, diterpene glycosides, lactones, flavonoids, and flavonoid glycosides. Whole plant leaves and roots are also used as a folklore remedy for different diseases in Asia and Europe . Andrographis paniculata has broad range of pharmacological effects including anticancer , antidiarrheal , antihepatitis , anti-HIV , antihyperglycemic, anti-inflammatory , antimicrobial, antimalarial, antioxidant, cardiovascular , cytotoxic and hepatoprotective properties.

Andrographis paniculata, a medicinal plant commonly used for symptomatic relief of the common cold, and its phytoconstituent andrographolide, have been repeatedly identified as potential antivirals against SARS-CoV-2 .*Andrographis* . *paniculata* is traditionally used as a bitter tonic for the treatment of diarrhoea. It can also be chewed with its juice concurrently applied on snake or insect bite wounds. In Ayurvedic medicine, *A. paniculata* is used for a wide range of ailments and diseases such as for pre- and post-natal care, dysmenorrhoea, malaria, and jaundice, as well as for cuts and wounds on the skin. Globally, *A. paniculata* is often used for relieving symptoms of common colds and upper respiratory tract infections. As the (COVID-19) pandemic worsens, with over 2 million related deaths by early 2021, the need for an effective treatment has become even more urgent. In January 2021, Thailand announced its pilot program for administering and investigating the effectiveness of *Andrographis paniculata* extract in patients diagnosed with COVID-19. Andrographolide has been listed as one of the main ingredients of a Traditional Chinese Medicine (TCM) known as *Xiyanping*, which has been recommended under the China National Health Commission treatment guidelines for COVID-19 based on TCM principle **[4]**

Bioinoculants are gaining importance globally because of their pollution free and environment friendly attributes. Microorganisms like AM fungi, *Azotobacter, Aspergillus awamori,* potassium solubilser are used as biofertilizers to enhance the growth and *Trichoderma viridae* is used as a biocontrol agent . However, the total Andrographis paniculata production (volume) through commercial cultivation is not exactly available in the literature. Due to its high demand, the herb is collected indiscriminately from the wild sources causing decline in the natural availability as well supply to the industry **[5].** The situation entails encouragement of the organic cultivation technology for the production of quality material curtailing excessive use of synthetic fertilizers and chemicals. Further, bio-inoculants add nutrients to the rhizosphere by natural processes like solubilising phosphorus (P) by phosphate solubilising bacteria (PSB) and arbuscular micorrhizal fungi (AMF), stimulating plant growth by providing growth promoting substances by plant growth promoting rhizobacteria (PGPR), fixing atmospheric nitrogen to plant available pools either by free living N fixers (Diazotrophs) or by symbiotic N fixers **[6].** The use of vermicompost can be utilized as plant growth media or soil conditioner **[7].** The response of application of bio-inoculants such as free living nitrogen fixing bacteria, plant growth promoting bacteria, and arbuscular mycorrhizal fungi (AMF) for improving growth, yield and usefulness for suppressing diseases of medicinal plants has also been studied **[8]**.

Since there is no much report on the use of different combinations of bioinoculants which can supplement NPK to maximize growth and biomass production without using synthetic fertilizers our main objective was to find out the suitable consortia of microbial inoculants for *Andrographis paniculata* and to study the synergistic effect on the growth and nutrient uptake.

MATERIALS AND METHODS

The seeds of *Andrographis paniculata* were collected from the western ghats region, the seeds were sown and seedlings were raised .Since the seeds are having dormancy, to break the dormancy of seeds , one set of seeds were treated with 1N HCl for 10 minutes followed by 0.1% GA_3 for 20 minutes. Another set of seeds were treated with 1N HCl for 10 minutes followed by 0.1% IBA for 20 minutes. Then the seeds were washed in water and sown in sterile soil containing sand, soil and compost in the ratio 1:1:1.After 20 days germinated seedlings were transplanted to different plastic pots for bioinoculant treatments. The experiments were conducted in a randomized replicated block design with 10 replicates for each treatment. Seeds were collected from the plants growing in the Western Ghats region. Seedlings were raised in pots containing sterilized soil and FYM(1:1). Experiments were carried out in a randomised replicate blocks with five replicates under greenhouse condition. After 20 days the seedlings were transplanted to plastic pots for bioinoculant treatments. The treatments included the bioinoculants;

T_1-*Glomus mosse* + *Azotobacter* +*Aspergillus awamori* + K solubiliser +*Trichoderma viridae*
T_2- *Glomus fasciculatum* + *Azotobacter* +*Aspergillus awamori* + K solubiliser +*Trichoderma Viridae*
T_3-*Glomus mosse*
T_4- *Glomus fasciculatum*
T_5- K solubiliser, *Frateuria aurentia*
C-Control

The Microorganisms viz., *Glomus mosse* , *Glomus fasiculatum, Azotobacter, Aspergillus awamori* , *Frateuria aurentia* were used to supplement NPK and a biocontrol agent *Trichoderma viridae*.For the first time *Frateuria aurentia* is used as a bioinoculant to study the K content of the plant after inoculation. The soil containing arbuscular mycorrhizal spores with the host roots was used as the inoculum for the seedlings of kalmegh. AM fungal inoculum treatment was repeated at 20,50 and 80 days after the first inoculation. Other bioinoculants were given in the form of liquid biofertilizer once in thirty days. Growth parameters like shoot length, root length, number of branches, number of leaves, fresh weight, dry weight were recorded at 90 days. Arbuscular mycorrhizal percent root colonization was determined by the method of Phlips and Hayman [**9**]. Washed roots were fixed in FAA for 4-10 hours. Then they were suspended KOH solution and autoclaved at 15 lb pressure and 121°C for 15 minutes, then KOH solution was decanted and neutralized with 1 per cent HCl for 5 minutes. The root bits were stained with 0.05 per cent trypan blue in lactophenol and number of vesicles and arbuscules were obnserved. Percent root infection by AM fungi was calculated .

Extramatrical chlamydospores produced by the mycorrhizal fungus were estimated by the wet sieving and decanting method outlined by Gerdeman and Nicolson [**10**] using sieves of various sizes.100 ml of representative soil samples from each treatment was suspended in sufficient quantity of water and stirred thoroughly. The resulting soil suspension was passed through sieves kept one below the other in the same order. The soil and the spores from the bottom of two sieves were transferred to a nylon mesh of the same size as the last sieve. The nylon mesh with spores was placed in a Petri plate and the spores were counted under stereomicroscope.

Estimation of nitrogen was done by the Micro-Kjeldahl method [**11**]**.**Nitrogen present in the sample was recorded by digesting the sample with concentrated sulphuric acid and mercuric sulphate was used as a catalyst. Ammonium sulphate was converted to ammonia by the addition of alkali and the liberated ammonia was subjected to absorption in 2 per cent boric acid and a mixed indicator of methyl red and methylene blue. Then the absorbed ammonia is titrated directly against 0.02 N H_2SO_4. Determination of N equivalent of sulphuric acid was done by using standard Nitrogen solution containing pure and dry ammonium sulphate. Then Percentage of Nitrogen was calculated.

The total phosphate in the sample was estimated by the following method as outlined by Jackson,1971[**12**]. Powdered and dried plant material was digested using 10 ml of triacid mixture containing concentrated nitric acid, 60 per cent perchloric acid and sulphuric acid in the ratio 10:4:11. To the digest vanadomolybdate reagent and 2N HNO_3 was added and allowed to react for 30 minutes to develop yellow colour. The per cent transmission was read at 420 nm wavelength and total phosphate was estimated by comparing the absorbance with a standard curve.

To estimate potassium in plant samples Toth and Prince ,1949 [**13**] method was used. Dried and powdered material was digested using tri-acid mixture and filtered. The filtrate was used for estimation of K. Plant samples were diluted by transferring 2 ml and 1 ml of test solution into separate 50 ml volumetric flask and

diluted to 50 ml mark with distilled water. Similarly blank test solution was also diluted. Diluted plant samples and blank test solution were atomised and the flame photometer readings were taken. Standard curve was obtained by using standard potassium solution made by using pure and dry potassium chloride. Then potassium estmation was carried out.

Estimation of calcium and magnesium was carried out in the following manner (Dewis and Freitas,1970[**14**]. Dried and powdered plant material was acid digested with triple acid mixture The volume of ash solution was made upto 100 ml with distilled water. From this 5ml of sample solution was taken in 250 ml conical flask,100 ml distilled water, 5 ml of 1N NaOH solution and 200 mg of muroxide indicator was added. This was titrated against 0.01 EDTA taken in the burette until the colour changes to purple at the end. Percent Ca was calculated

Magnesium was determined indirectly from the same solution from which calcium has been estimated as the difference between the calcium plus magnesium reading and calcium reading alone. 5 ml of the sample solution was taken in a 250 ml conical flask .To this 100 ml distilled water, 15 ml buffer solution and 7.5 ml of 1N NaOH solution was added. Erichrome Black-T was added as indicator and titrated against 0.01M EDTA solution taken in burette and end point was blue. This reading gives both Ca and Mg. Then Percent Mg was calculated using the formula.

Estimation of sodium was done by the method as outlined by Toth,1948 [**15**]. 5 meq of NaCl solution was prepared. Using this different sodium standards were prepared. Then calibration of the flame photometer and testing of the plant sample test solution was done. Standard graph for sodium was prepared. Using this, concentration of sodium was determined.

The protein content in the extract was estimated by the method outlined by Lowry,1951[**16**] using Folin Ciocalteau reagent. The O.D. was recorded at 640 nm. The amount of total proteins was determined using BSA as standard.

Quantitative estimation of carbohydrates was done by anthrone method . 0.1 mg of the plant sample was homogenized in 80 per cent methanol. The homogenate was centrifuged. The supernatant was retained. To 1ml of the supernatant freshly prepared anthrone reagent was added and boiled in water bath. The mixture was cooled and the change in colour from green to deep green was read at 630nm using spectrophotometer. The blank was distilled water with anthrone reagent. Glucose was taken as standard (500µg/ml**)**.
Estimation of reducing sugars was carried by the method as outlined by Mahadevan and Sridhar ,1982 [**17**] Plant sample was homogenized in 80 per cent ethyl alcohol. The homogenate was centrifuged and the supernatant was collected. To 1ml of the supernatant DNS (Dinitrosalicylic acid) was added and the reaction mixture was heated in boiling water bath. After orange yellow color development, 40 per cent Rochelle-salt solution (Sodium potassium tartarate solution) was added as the contents of the tube were still warm. Later tubes were cooled under running tap water. O.D. values were recorded at 540nm. Glucose was taken as standard.

RESULTS

Cent percent germination was observed in seeds treated with 1N HCl for 10min and 0.1%GA_3 for 20min whereas 1N HCl (10min) and 0.1% IBA(20 min) showed 95% germination. Viability of seedlings was maximum in 1N HCl and 0.1% IBA treated seedlings.
Combined inoculation with T_2- *Glomus fasciculatum* + *Azotobacter* +*Aspergillus awamori* + *Frateuria aurentia* + *Trichoderma viridae* showed significant increase in plant height of kalmegh followed by T5 that is *Frateuria aurentia* and T_1-*Glomus mosse* + *Azotobacter* +*Aspergillus awamori* +*Fraturia aurentia*r +*Trichoderma viridae* compared to single inoculation and uninoculated control.
The plants inoculated with T_1 showed significant increase in number of branches, and number of leaves of the plants. T_5 was found to be the second best with respect to number of branches and T_2 with respect to number of leaves.T_2, T_3 and T_4 treated plants did not respond well with respect to number of branches followed by control. Number of leaves was more in T_5 and T_4 treated plants compared to control but T_3 treated plants showed least number of leaves. Fresh weight of the plants is significantly high in T_2 plants followed by T_5 and T_1. Fresh weight of T_3 and T_4 plants was found to be lower than control. There was a significant increase in the dry weight of the plants which were given T_2 treatment, second best was T_5 followed by T_1. Lowest dry weight was observed in T_3 plants followed by T_4 compared to control.
At 90 days percent root colonization was maximum and similar in T_1 and T_2 plants but spore number was maximum in T_2 plants. Maximum number of arbuscules and vesicles were found in T_2 plants. Less AM colonization was observed in T_4 plants and T_3 plants. No colonization was observed in plants inoculated with *Frateuria aurentia* (T_5) and control.

Nitrogen content was maximum in T2 plants followed by T1 plants. The N content was significantly higher in these plants compared to other treated plants and uninoculated control. P content was significantly high in T_2

plants compared to other treatments and uninoculated control. Second best was T_1 plants followed by T_4 plants. Even in T_3 and T_5 Plants P content was more than control. In both T_2 andT_1 plants K content was maximum compared to uninoculated control. Next best was T_5 followed by T_3 and T_4 , the uninoculated control showed least content.
Ca content was significantly high in T_2 plants followed by T_1 and T_5 plants.T_3 and T_4 plants showed slightly higher Ca content compared to uninoculated control. Both T_1 and T_2 plants showed maximum Mg content followed by T_5 plants compared to T_3 , T_4 and uninoculated control. Sodium content was same in bioinoculant treated and uninoculated control which shows that bionoculants may not help the plants in sodium uptake.
T_2 plants recorded significant increase in protein content, total carbohydrate content and T1 showed similar total carbohydrate content. Both T1 and T2 plants recorded maximum reducing sugar content.

DISCUSSION

Treatment of seedlings with a consortia of microbes which could supplement NPK resulted in better growth of the plants compared to control. Shubha and Anusuya ,2010[**18**] reported significant increase in the growth parameters, nutrient and antioxidant content in bioinoculant treated micropropagated *Costus pictus*. Maximum shoot length and fresh weight was observed in plants with combined inoculation of *Glomus fasciculatum, Azotobacter,Aspergillus awamori, K solubiliser* and *Trichodrema viridae*.
Maximum number of branches,leaves and dry weight were observed in *Glomus mosse, Azotobacter,Aspergillus awamori, K solubiliser* and *Trichodrema viridae* treated plants .Many authors have reported increased dry weight when AM fungi was inoculated with microbes like *Azotobacter,Trichoderma*.[**19**] P.content was maximum in T_1 plants. This upholds the observations made by earlier workers that Phosphorus uptake by AM fungus is increased when it is coinoculated with PGPR, *Azotobacter* **[20].**Trichoderma ,maximum root length was observed implants inoculated with K solubiliser. Protein content was maximum in T_1plants.Maximum root colonization was observed in T_1 and T_2 plants.
Treatment of seedlings with a consortia of microbes which could supplement NPK resulted in better growth of the plants compared to control. Maximum shoot length and fresh weight was observed in plants with combined inoculation of *Glomus fasciculatum, Azotobacter,Aspergillus awamori, K solubiliser* and *Trichoderma viridae*(T_2). Aparna,2000 [**21**]reported significant increase in plant height in *Apaniculata* when treated with *Glomus mosse* and *Trichoderma harzianum* than uninoculated control.

T_1 inoculated plants showed maximum number of branches and leaves. Gururaja reported improved plant growth,plant height,stem girth,number of leaves and stem dry weight and N and P content when Sunflower plants were inoculated with *Glomus fasciculatum ,Azotobacter chroococcum* and *Penicillium glaucum*.*Frateuria aurentia* (T_5) treated plants also showed significant improvement with respect to number of branches and leaves.

Both fresh weight and dry weight was significantly higher in T_2 plants. But T_3 and T_4 showed least biomass compared to control. Inoculation of medicinal plants with PGPR has improved plant growth and biomass in *Piper nigrum* ,Neem and Turmeric . AM colonization studies showed that though both T_1 and T_2 plants showed maximum colonization, number of arbuscules and vesicles were maximum in T_2 plants showing the synergistic interaction with PGPR and must be responsible for improved growth and biomass.

Maximum root length was observed in plants inoculated with K solubiliser (T_5). Hence *Frateuria aurentia* is a good root growth promoting bacteria.

P content was significantly higher in T_2 plants followed by T_1. This upholds the observations made by earlier workers that Phosphorus uptake by AM fungus is increased when it is coinoculated with PGPR such as *Azospirillum***[22],** *Azotobacter* , Trichoderma **[23].** Even the plants inoculated singly with *Glomus mosse* (T_3) and *Glomus fasciculatum* (T_4) showed more P content compared to T_5 and control which upholds the fact that AM fungi are good P solubilisers.
Potassium content was significantly higher in both T_1 and T_2 plants which may be due to synergistic interaction among the bioinoculants. K solubiliser also gave good results. According to the results *Frateuria aurentia* is a good K solubiliser but its synergistic interaction with other bioinoculants used gave even better percentage of K in kalmegh.
Mineral elements like Ca and Mg showed higher in T_2 plants followed by T_1 which again showed that T_2 is a better combination for kalmegh followed by T_1.Srinivasa [**24],** reported that the dual inoculation of *Glomus macrocarpum* and *Pseudomonas striata* significantly increased plant nutrients that is uptake of P,Cu,Zn,Mn, and Fe and plant dry weight. There was no significant difference between uninculated control and other treatments. But, surprisingly there was no difference in the sodium content of the treated and uninoculated control plants.[**25**]
Cultivation of *Andrographis paniculata* has started recently on a commercial scale. Inoculation with AM fungi and plant growth promoting rhizo microorganisms can help in the yield and in turn help in reducing the chemical fertilizer application and thus the cost of cultivation and protecting the environment and soil health.

TABLES

Table – 1, Synergistic effect of bioinoculants on number of vesicles, arbuscules, per cent root colonization, spore number in rhizosphere soil of *Andrographis paniculata* (Burm.f) Nees. at 90 days.

Treatments	No. of arbuscules	No. of vesicles	Per cent root infection	No.of spores in 25 ml of rhizosphere soil
T_1	91*	112*	90*	88*
T_2	107*	135*	90*	94*
T_3	37*	49*	60*	81*
T_4	73*	141*	80*	71*
T_5	0	0	0	0
C	0	0	0	0

*Significant at P < 0.05

Table – 2. Growth parameters of *Andrographis paniculata* (Burm.f) Nees. As influenced by bioinoculants (90 days)

Treatments	Shoot length (cm)	Root length (cm)	No. of branches	No. of leaves
T_1	20.1*	8.1	15*	80*
T_2	25*	10*	5	76*
T_3	10	7	4	22
T_4	12.6	6	6*	50*
T_5	23.6*	10.5*	13*	67*
C	9.8	6.1	3	25

*Significant at P < 0.05

Table 3 : Influence of bioinoculants on plant biomass of *Andrographis paniculata* (Burm.f) Nees. (90 days) (Values expressed in grams for fresh weights and dry weights)

Treatments	Plant fresh weight	Plant dry weight
T_1	2.1*	0.404*
T_2	2.5*	0.461*
T_3	0.9	0.085
T_4	1.01	0.16
T_5	2.2	0.431*
C	1.2	0.21

*Significant at P < 0.05

Table – 4 : Influence of bioinoculants on NPK content of *Andrographis paniculata* (Burm.f) Nees. (90 days) (Values expressed in per cent)

Treatments	Nitrogen	Phosphorus	Potassium
T_1	4.2*	0.37*	0.337 *
T_2	4.5*	0.41*	0.337 *
T_3	3.4*	0.3*	0.249

T_4	3.3	0.35*	0.24
T_5	3.3	0.29	0.324 *
C	3.2	0.18	0.193

*Significant at P < 0.05

Table – 5 :Influence of bioinoculants on Ca, Mg and Na content of *Andrographis paniculata* (Burm.f) Nees. (90 days) (Values expressed in percent for Calcium, Magnesium and ppm for SodiuM)

Treatments	Calcium	Magnesium	Sodium
T_1	0.56112*	0.245*	0.8
T_2	0.64128*	0.245*	0.8
T_3	0.4008	0.147*	0.8
T_4	0.32064	0.098	0.8
T_5	0.48096*	0.196	0.8
C	0.24048	0.049	0.8

*Significant at P < 0.05

Table – 6 : Influence of bioinoculants on Protein, Total Carbohydrates and Reducing sugar content of *Andrographis paniculata* (Burm.f) Nees. (90 days), (Values for protein, Total Carbohydrates and Reducing sugars expressed in mg g^{-1})

Treatment	Protein	Total Carbohydrate	Reducing Sugars
T_1	4.9	6.9*	1.5
T_2	5.4*	6.6*	1.5
T_3	4.3	4.8	1.3
T_4	4.1	4.2	1.2
T_5	4.8	4.2	1.2
C	3.9	3.8	1.1

*Significant at P < 0.05

CONCLUSION

As the plant is critically endangered and is in great demand a consortia of microbes viz., *Glomus fasciculatum* + *Azotobacter* +*Aspergillus awamori* + K solubiliser +*Trichoderma viridae* can be effectively used to get better growth and yield of *Andrographis paniculata* and thereby reducing the exploitation of the plant from the wild and conserving them in their natural habitat. The study has also proved that the K solubiliser, *Frateuria aurentia* is a potential bioinoculant for Kalmegh alone and in combination with respect to potassium uptake of the plant.

REFERENCES

1. 1.Nor Elliza Tajidin , Khozirah Shaari , Maulidiani Maulidiani et al (2019) Metabolite profiling of Andrographis paniculata (Burm. f.) Nees. young and mature leaves at different harvest ages using 1H NMR-based metabolomics approach.9(1):16766
2. Calabrese ,C., Berman,S.H., Babish,J.G., Ma X, Shinto,L., Dorr,M.K., Wells Wenner ,C. A., Standish, L. J.,(2000). A phase I trial of andrographolide in HIV positive patients and normal volunteers .Phytotherapy research ,14**:**333-338.
3. M.Rajannaa ,B.Bharathi ,B.R.Shivakumara, M.Deepak et al (2021) Immunomodulatory effects of Andrographis paniculata extract in healthy adults – An open-label study. volume 12 issue 3: 529-534.

4. 4.Xin Yi Lim, Janice Sue Wen Chan, Terence Yew Chin Tan et al (2021) Andrographis paniculata (Burm. F.) Wall. Ex Nees, Andrographolide, and Andrographolide Analogues as SARS-CoV-2 Antivirals? A Rapid Review. volume 16. issue 5.
5. Shi-Lin Chen, Hua Yu, Hong-Mei Luo et al (2016),Conservation and sustainable use of medicinal plants: problems, progress, and prospects. Chin Med. 2016; 11: 37.
6. Hamdi Hussein Zahran (1999) Rhizobium-Legume Symbiosis and Nitrogen Fixation under Severe Conditions and in an Arid Climate. 63(4): 968–989.
7. Alagesha boopathi, C. and Ramachandran . V. S. 2000**.** Methods of Mass-Multiplication of Indian *Andrographis* Wallich Ex Nees and its chemical analysis" Journal of Economic and Taxonomic Botany . Vol. .24 . No . 3 . 659-662.
8. 8.Singh Umrao., Wadhwani, A. M. and Johri, B. M**.**1983. *Andrographis paniculata*.Dictionary of Economic Plants, Second Edition.Indian Council of Agricultural Research., NewDelhi. 16.
9. Philips,J.M., and Hayman,D.S., 1970.Improved procedure for clearing roots and staining
10. parasitic fungi and VAM mycorrhizal fungi for rapid assessment of infection. Transactions of. British mycolological Soc., 55 :158-161
11. Gerdemann ,J.W. and Nicolson *, T.H 1963*, Spores of mycorrhizal endogone species extracted from the soil by wet seiving and decanting, Transactions of British Mycological Society, 46 ,235-244.
12. Chopra, R.N., Nayar, S.L. and Chopra, I.C,.1986. *Andrographis* .Glossary of Indian Medicinal plants, Council of Scientific and Industrial Research. 18 .
13. Jackson ,M.L. 1971 , Soil Chemical Analysis, Prentice Hall of India (p) Ltd. New Delhi
14. Toth, S.J., Prince, A.L., Wallace, A. and Mikkelsen, D.S.1949. Rapid quantitative determination of eight mineral elements in plant samples by a systematic procedure involving use of a flame photometer. Soil Science. 66;459-466.
15. Dewis, J. and Freitas, F. (1970) Physical and chemical methods of soil and water analysis. FAO Soils Bulletin 10. FAO, Rome.
16. S.J Toth, A.L Prince et al, (1948) Rapid quantitative determination of eight mineral elements in plant tissue by a systematic procedure involving use of a flame photometer. volume 66 . issue 6. 459-466
17. O.H. Lowry, N.J. Rosebrough et.al (1951) Protein measurement with the folin Phenol Reagent. 193(1):256-275
18. A. Mahadevan and R. Sridhar, "Methods in Physiological Plant Pathology," 2nd Edition, Sivakami Publications, Madras, 1982.
19. Shubha and Anusuya, D. 2010.Antioxidant and nutrient content of micropropagated Costus pictus inoculated with beneficial soil microorganisms. Advances in plant sciences.23(02):351-354.
20. 19.Bagyaraj,D.J. and Menge, J.A. 1978.Interaction between a VAM and Azotobacter and their effects on the rhizosphere microflora and plant growth. New Phytol.80 567-573
21. Meenu,R. and Saralabai, V.C. 2001.Effect of VAM and Nitrogen fixing biofertilizers on growth in Sorghum vulgare.Advances in Plant Sciences . 14(1) 615-617.
22. Aparna,J.(2000), Mycorrhizal association in medicinal plants and response of kalmegh (Andrographis paniculata Nees) to VAM and a plant growth promoting rhizomicroorganism. M.Sc. thesis submitted to UAS Bangalore.
23. Tilak, K.V.B.R. and Singh, C. S. 1988.Response of pearl millet(pennisetum americanum) to inoculation with Inoculation with Vesicular Arbuscular Mycorrhizae and Azospirillum brazilensewith different sources of phosphorus. Current Sceince.57. 43-44.
24. Earanna ,N., Tanuja, B.P., Bagyaraj,D. J. and Suresh,C.K**. (**2002).Vesicular Arbuscular Mycorrhizal selection for increasing the growth of *Rauwolfia tetraphylla*. Journal of Medicinal and Aromatic Plant Sceinces.24.695-697.
25. Srinivasa,M. N. and Krishnaraj,P.V. (1992**).** Synergistic interaction between VA mycorrhizal fungi and phosphate solubilising bacterium in chilli(*Capsicum annum).* Zentralblatt fur Mikrobiologie, 147:126-130.
26. Patra, D. D., Chattopadhyay, A., Ashok , k.,Singh., Tomar., Aparbal singh, Mishra,H.O., Alam. M. and Khanuja,S.P.S**.**2004. Agrotechnology of kalmegh(*Andrographis paniculata)* Journal of Medicinal and Aromatic plant Sciences. 26. 534-537.