



BIOMASS YIELD, NUTRIENT UPTAKE AND CHEMICAL CONSTITUENTS OF *WITHANIA SOMNIFERA* (L.) DUNAL AND *ADHATODA VASICA* NEES MEDICINAL VALUED SPECIES INFLUENCED BY MICROBIAL INOCULATION

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Abstract

AMF, PSB and *Azotobacter* microbial consortia inoculated singly and in-combinations in *Withania somnifera* (L.) Dunal and *Adhatoda vasica* Nees. The combined AMF+PSB found best in *W. somnifera* increase yield by 1.56 folds compared to uninoculated plants while in *A. vasica* tripartite inoculants AMF+PSB+*Azotobacter* rendered more than 2.0 folds higher yield. Treatment responsiveness observed highest in AMF+PSB (T4)> AMF+*Azotobacter* (T5)> Tripartite inoculants (T7)> PSB (T2)> PSB+*Azotobacter* (T6)>AMF (T1)> *Azotobacter* (T3) in *W. somnifera*. In contrary, the responsiveness of treatment in *A. vasica* was highest with tripartite inoculants (T7)> PSB+ *Azotobacter* (T6) > AMF+ *Azotobacter* (T5)> AMF+PSB (T4)> single inoculants. Single strain microbial exhibited no response in *A. vasica* when compared to control plant at $P<0.05$. Nutrient uptake in plant also improved significantly at $p\geq 0.05$ due to microbial inoculations in both the test species however P uptake was more than N and K uptake. Overall, N and P uptake enhanced between 15.71-33.80% and 52.14-64.28% respectively in *W. somnifera* and 2.63-50% and 60.83-133.33% in *A. vasica* due to inoculations compared to control plants. The protein, carbohydrate and polyphenol content also augmented upto 36.20%, 89.53% and 266.23% respectively in *W. somnifera* under AMF+PSB and 50.0%, 95.61% and 29.60% respectively in *A. vasica* with tripartite microbial inoculation.

Key words: Medicinal plants, Biomass yield, Nutrient uptake, biochemical constituents, A-Mycorrhiza, *Azotobacter*.

Introduction

Medicinal plants are the most important source of life saving drugs for the majority of the World population. There are numerous plants that have a long history of curative properties against various diseases and ailments. For millennia, these plants have been a valuable source of therapeutic agents and still many of today's drugs are plant-derived natural products or their derivatives (Newman and Cragg, 2012). Furthermore, an increasing reliance on the use of medicinal plants in the societies has been traced to the extraction and development of several drugs from these plants. Medicinal plants are of great interest to the researchers as most of the drug industries depend, in part, on plants for the production of pharmaceutical compounds (Choudhari and Trivedi, 2011). Since ancient time, herbal drugs are integral part of kitchen of Indian houses and currently about 8610 licensed herbal

units, thousands of cottage level unregulated herbal units and millions of folk healers are dependent on medicinal plants for traditional healthcare practice, Ayurvedic, Unani, Siddha and swa-rigpa (NMPB, 2018).

Generally Forest is a natural home for most of the medicinal plants which is exploited heavily for the use at pharmaceutical industries. The consolidated demand of commercial drugs in India estimates 512,000MT out of which 134000MT export, 195000MT used in domestic units and about 167500MT medicinal herbs are consumed every year by households (NMPB, 2018). Total 1178 medicinal plant species are in the trade practice in which 242 plants are used annual quantities of more than 100MT. The over exploitation of these resources results in a short supply and many of them become vanished from forest area. Due to increasing gap in the demand and supply the price of medicinal plants has jumped and has opened a new door to farmers for its cultivation as other

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agricultural crops. The area of medicinal crops cultivation in India has increased from 262000ha during 2005-06 to 633900ha in 2015-16 and similarly, the production has increased from 2.02MT to 10.22MT respectively during aforesaid periods with an annual growth rate of 2.76% per annum (Chowti *et al.*, 2018). These indicate that we are in mode from collection to cultivation as now a day many important medicinal plants are being cultivated by farmers as an progressive agriculture (Chatterjee, 2001 and Rajeswara Rao, 2010).

Though the cultivation of medicinal plants now become remunerative and popular among farmers due to increasing gap between demands and supply, but constraints not to use any chemical fertilizers in such crops effects yield of medicinal species. Usually chemicals are prohibited in medicinal crops due to fear of contamination and degradation in chemical constituents and value of plant products (Alori and Babalola, 2018). But currently agriculture soil has become addict of chemicals and now we can't think cropping without chemicals (fertilizers, pesticides, herbicides etc.) though we know its deleterious effect in plant products, soils and on the health of producer and consumer. In addition, chemicals are prohibited in medicinal plants as it is used by patient for curing disease and as a health tonic and the use of any chemical may be harmful to consumer. Therefore, it is paramount need to find out other ecofriendly sources of plant nutrition especially for medicinal plants and for sustainable production of medicinal plants outside forest for concurrent supply of raw materials for ever increasing demands worldwide.

Currently the application of micro-inoculants increased as a potential alternative to chemical fertilizers and pesticides, particularly in developing countries owing higher yields. This inoculum comprise nitrogen fixers, phosphate solubilizers and AMF (*Arbuscular mycorrhizal fungi*) capable of improving growth and yield (Alori and Babalola, 2018), essential oil and its constituents, carbohydrates, soluble sugars and nutrients contents (Pathak and Kumar, 2016) when inoculated either through seed, soil and roots (Vessey, 2003; Ahmed and Kibret, 2014). These augment disease and drought resistance in plants by secreting enzymes and hormones through physiochemical activities in plants (Hynes *et al.*, 2008; Russo *et al.*, 2008). Micro-inoculants improve the nutritious properties by increasing the antioxidant activity and the total phenolic compounds and chlorophyll (Khalid *et al.*, 2017). More importantly, micro-inoculants with highly concentrated microbial populations symbiotically or free living persist near root, influence plant growth and health which effectively ameliorate soil quality and

fertility (Hassan, 2017). In view of this, the study of micro-inoculants to cultivation of medicinal valued species may be highly beneficial not only in increasing the yield and quality of medicinal plant products but also for the promotion of environmental friendly technology among farmers. As the cultivation of medicinal plants is not an old practice, the standardized modules are lacking compared to agriculture (Ahmed and Kibret, 2014) and thus the reports of micro-inoculant on medicinal plant species are also very limited. In present study, three isolates *viz* AMF, PSB and *Azotobacter* have been experimented in two most widely used medicinal plants *Withania somnifera* (L.) Dunal and *Adhatoda vasica* Nees to evaluate their responsiveness under nursery condition on parameters of biomass yield, nutrient uptake and certain chemical constituents.

Materials and Methods

The medicinal plants selected in present investigation are highly important not only in traditional medicine but also in the formulations of modern drugs. *Withania somnifera* (L.) Dunal a widely cultivated medicinal valued species belongs to family Solanaceae and is known for its rejuvenative and restorative properties and referred as Indian ginseng. It is indigenous to India and cultivated widely in central and southern part of India (Kothari *et al.*, 2003; NMPB, 2018). Roots are used to cure hiccups, cough, dropsy rheumatism and male disorders, inflammation, ulcers and scabies in the form of external application. It is been in use for a very long time for all age groups and both sexes and even during pregnancy without any side effects. It is useful adjunct for patients undergoing radiation and chemotherapy. Recently *W. somnifera* has used to inhibit the development of tolerance and dependence on chronic use of various phytotropic drugs (Gupta and Rana, 2007).

Adhatoda vasica Nees belongs to family Acanthaceae is commonly known as Malabar Nut, distributed throughout India up to an altitude of 1300m. The leaves, flowers, fruits and roots are extensively used for treating cold, cough, whooping cough, chronic bronchitis and asthma as sedative, expectorant and antispasmodic in traditional medicines and also at household level. It is also used by traditional midwives at the time of delivery (Kumar *et al.*, 2013) for reducing pain. The plant has been reported to have high medicinal values (Rachna *et al.*, 2011). Considering the importance of these two medicinal plants the present study was undertaken at forestry nursery of Guru Ghasidas Vishwavidyalaya, Bilaspur, Chhattisgarh lies within latitude 21°47' to 23°8' and longitude 81°14' to 83°15'.

Seeds of both the medicinal plants were shown in polythene bags (size 12 × 23.5cm) filled with 1:1:0.25 ratio Sand Soil and FYM (Farm yard manure). Before filling the mixture it was autoclaved at 15 Psi for 2 hour. After one month of DOS (Day of Sowing) thinning was done and only one seedling remained in each pot.

AMF, (*Arbuscular Mycorrhizal Fungi*) dominated with *Acaulospora scrobiculata*, *Glomus funneliformis* and *Acaulospora* species isolated from natural Teak (*Tectona grandis*) of dry forest area was decanted (Gerdemann and Nicolson, 1964) mass multiplied using *Cymbopogon* grass as trap (Douds *et al*, 2005). 20g inocula consist 0.17×10^4 infective propagules alongwith 10g of >80% AMF colonized roots of trap plant was inoculated in rhizosphere in 30 day aged *W. somnifera* and *A. vasica*. Similarly the PGPR (Plant growth promoting rhizobacteria) micro-inoculants were placed in holes 0-20cm depth made around rhizosphere of seedling followed by refilling of sterilized soil into hole.

Starter inoculum of PSB (Phosphorus Solubilizing Bacteria-*Pseudomonas straita*) and *Azotobacter chroococcum* strain procured from TCB college of Agriculture and Experimental Station (Indira Gandhi Krishi Vishwavidyalaya) Bilaspur were used @ 20g per plant as per experimental design.

Experiment was arranged in randomized block design consisted total 7 treatments (T1- AMF, T2 - PSB, T3 - *Azotobacter*, T4 - AMF+PSB, T5 - AMF+*Azotobacter* T6 - PSB+*Azotobacter*, T7 - AMF+PSB+*Azotobacter*) excluding the control without inoculation. Each treatment consist 5 replicates with 5 plants in a block.

Observations recorded after 5 months of plant age. The dry biomass yield of plant was determined by keeping plant in hot oven at 80°C for 3 days. The responsiveness/efficacy of different microbial inoculant was calculated as following:

$$\text{Responsiveness (\%)} = \frac{\text{Biomass of inoculated plant} - \text{Biomass of uninoculated plant}}{\text{Biomass of uninoculated plant}} \times 100$$

The leaf were dried and powdered sample 50 mg each was macerated with 20 ml of ethanol and centrifuged (1200 rpm) for 15 minute, the supernatants were removed and were concentrated on a water bath. The volume was made up to 50 ml with distilled water and processed further for soluble sugars. One ml aliquot of each of the test sample was used to quantifying the total levels of carbohydrates using phenols H₂SO₄ method (Dubois *et al.*, 1951). Protein content was determined by the procedure described by Lowry *et al.*, (1951). One gram of leaf sample was ground with 5 ml of phosphate buffer (pH₇) and centrifuged at 3000 rpm for 20 minutes. 3ml

extract was taken in 3ml of 20% tri-chloro-acetic acid and kept in water bath for 20 minutes and again it was centrifuged at 3000 rpm for 20 minutes. Pellet was collected and washed with 6 ml of acetone then pellet allowed to dissolve in 5ml 0.1 NaOH and mixed well. It was kept for 10 minutes and 0.5 ml of Folin was added. It was incubated for 30 minutes in dark and the absorbance was read at 660 nm using spectrophotometer. The amount of protein was estimated using Bovine serum albumin as the standard. Polyphenol in plant was also estimated as per Schanderi, (1970).

Estimation of nutrient content in plant tissues was done after digestion of sample in tri-acid mixture for 2 hour at 425°C. The aliquat was used for estimation of macro and micro nutrients in plant. Total N was determined using auto-kjeltech, 2300. Estimation of phosphorus was done using UV spectrophotometer and by K flame photometer. Fe, Mg, Zn in plant determined by Atomic Absorption Spectrophotometer.

All the data taken from 5 replicates were subjected to statistical analysis by using SPSS.

Results and Discussion

Treatments increased root biomass, shoot biomass and total dry biomass yield of studied medicinal valued species significantly at $p \geq 0.05$ (Fig. A, B, C and D), however the application of single micro-inoculant could not rendered apparent result in *A. vasica* when compared with the control plants. Root biomass was highest with PSB and AMF+PSB+*Azotobacter* in *W. somnifera* and *A. vasica* respectively. In contrary shoot biomass obtained highest with AMF+PSB and with tripartite inoculant in *W. somnifera* and in *A. vasica* respectively. Mean root biomass with dual inoculation enhanced by 2.41 folds and 1.03 folds in *W. somnifera* and *A. vasica* respectively compared to un-inoculated plants. Similarly, mean shoot biomass yield improved by 1.11 folds and 0.53 folds *W. somnifera* and *A. vasica* respectively due to coexistence of couple of micro-inoculants compared to plants without any inoculants (Fig. A and B). When comparing between dual inoculants and single microbial inoculants, root and shoot yield of plants with dual microbes was 28.57% and 57.54% higher respectively in *W. somnifera* and 73.7% and 40.8% higher in *A. vasica*. Moreover, the increment in total biomass yield was highest 156.7% and 203.13% due to inoculation of AMF+PSB and tripartite microbes in *W. somnifera* and *A. vasica* respectively compared to plant without any inoculant. Attribute specific variations existed in both the medicinal species due to varied response of microbial consortiums however the overall results found positive (Fig. C and D). These results are

in agreement with those of Das *et al.*, (2007) on *Stevia rebaudiana* and Gendy *et al.*, (2012) on Roselle plants that showed that the use of biofertilizers promotes root shoot growth and yield. Further the augmentation on plant growth parameters depends upon the presence of efficient inoculants as well as its compatibility with coexisting microbes to show synergistic relationship with hosts (Al-Fraihat *et al.*, 2011). Moreover, Walker *et al.*, (2003) concluded that plant secretes exudate via root helps in attracting soil microbial communities' results the uptake higher quantity of water and nutrients. As, the composition of root exudates varies upon plant species and microorganisms (Kang *et al.*, 2010) results different growth rate and biomass yields the degree of benefits of microbial inoculants also varied in plants as in case of present study. The responsiveness in *W. somnifera* showed trend highest with AMF+PSB (T4) > AMF > *Azotobacter* (T5) > Tripartite inoculants (T7) > PSB (T2)

>PSB+*Azotobacter* (T6) > AMF (T1). While the responsiveness trend was highest with tripartite inoculant (T7) in *A. vasica* follows with >T6 >T5 >T4 >T3 >T1 >T2 (Fig. C and D). The results could be attributed to the influence of AMF, PSB and *Azotobacter* inoculants as also reported by several other workers in agricultural crops due to multiple mode of action and factors such as synthesis of higher amount of growth promoting hormones (Spaepen and Vanderleyden, 2011), root exudates (Dakora and Phillips, 2002) and siderophores production (Rajkumar *et al.*, 2010) that enhance rooting capacity and root hairs which eventually ameliorated the nutrient uptake from the rhizosphere soil (Rodriguez and Fraga, 1999; Revilas *et al.*, 2000). Our findings also concord with many investigators pronounced the positive effects of *Azotobacter*, PSB and AMF (Hussain *et al.*, 2001; Chandra, 2015; El Hindi and El-Boraie, 2005; Ahemad and Kibret, 2014).

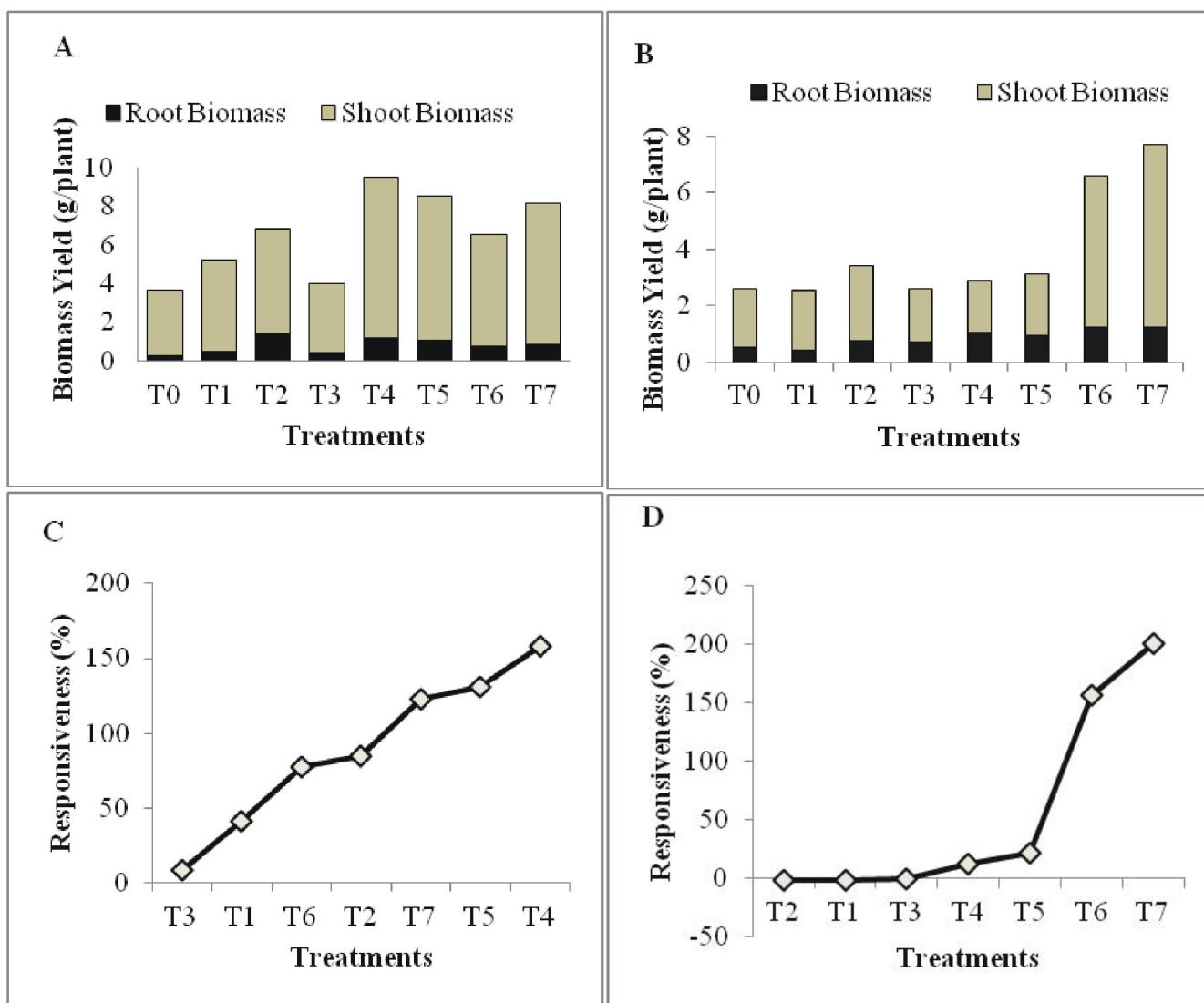


Fig. (A) Biomass of *W. somnifera* (B) biomass of *A. vasica*, (C) Responsiveness (%) of *W. somnifera* and (D) Responsiveness (%) of *A. vasica* as influenced by different bio-inoculants.

Table 1: Effects of different bio-inoculants on N,P and K contents of *W. somnifera* and *A. vasica* under nursery condition.

| Treatment | <i>Withania somnifera</i> | | | <i>Adhatoda vasica</i> | | |
|-----------------------------|---------------------------|------|-------|------------------------|------|------|
| | N(%) | P(%) | K(%) | N(%) | P(%) | K(%) |
| Control | 2.10 | 0.14 | 1.56 | 1.90 | 0.12 | 1.42 |
| AMF | 2.56 | 0.21 | 1.58 | 1.94 | 0.22 | 1.91 |
| PSB | 2.57 | 0.22 | 1.76 | 2.03 | 0.16 | 1.82 |
| <i>Azotobacter</i> | 2.16 | 0.21 | 1.59 | 1.90 | 0.20 | 1.71 |
| AMF+PSB | 2.85 | 0.24 | 1.86 | 2.26 | 0.23 | 1.71 |
| AMF+ <i>Azotobacter</i> | 2.63 | 0.21 | 1.84 | 2.82 | 0.19 | 1.79 |
| PSB+ <i>Azotobacter</i> | 2.61 | 0.20 | 2.24 | 2.33 | 0.22 | 2.01 |
| AMF+PSB+ <i>Azotobacter</i> | 2.81 | 0.23 | 2.31 | 2.85 | 0.28 | 2.43 |
| CD at $p \geq 0.05$ | 0.09 | 3.50 | 14.06 | 0.11 | 2.36 | 0.33 |

Data shown in table 1, depicts that the nutrients content improved significantly with the inoculation of microbial consortium compared to un-inoculated plant at $p \geq 0.05$, however result varied with different treatments. N and P content were 35.71% and 71.42% higher in *W. somnifera* with co-inoculation of AMF+PSB over control, while there were highest 50%, 133.33% and 71.12% increment in NPK respectively in *A. vasica* due to presence of tripartite inoculants consisted AMF+PSB+*Azotobacter*. It was observed that improvement in plant biomass also tend to ameliorate nutrient uptake in both the tested species might be due to positive relationship between biomass and nutrient content in plants. The data clearly indicated that the P uptake influenced more compared to N and K in plants as the mean increment ranged 15.71-33.80% N, 52.14-64.28% P in *W. somnifera* and 2.63-50% N, 60.83-133.33% P in *A. vasica* with different treatments compared to control. Similarly K content also found to increase in the treated plant comparing control. It may be remarked that inoculating the plants with micro-inoculants tended to a significant increase in biomass due to higher uptake of N, P and K. The uptake of Fe and Zn also increased due to the presence of beneficial microbes in rhizosphere of both the plants. It was proved from the data (Table 2)

Table 2: Influence of AMF, PSB and *Azotobacter* in different combinations on Fe and Zn contents of *W. somnifera* and *A. vasica*.

| Treatment | <i>Withania somnifera</i> | | <i>Adhatoda vasica</i> | |
|-----------------------------|---------------------------|------|------------------------|------|
| | Iron | Zinc | Iron | Zinc |
| Control | 279 | 40 | 358 | 69 |
| AMF | 507 | 42 | 327 | 45 |
| PSB | 503 | 44 | 436 | 91 |
| <i>Azotobacter</i> | 471 | 41 | 345 | 54 |
| AMF+PSB | 665 | 75 | 332 | 96 |
| AMF+ <i>Azotobacter</i> | 576 | 62 | 390 | 97 |
| PSB+ <i>Azotobacter</i> | 587 | 56 | 460 | 97 |
| AMF+PSB+ <i>Azotobacter</i> | 627 | 60 | 517 | 108 |
| CD at $p \geq 0.05$ | 13.0 | 9.0 | 15.22 | 11.0 |

that the Fe and Zn contents in plant increased highest 138.35% and 87.5% respectively in AMF+PSB treated *W. somnifera* compared control plant, while that was 44.22% and 56.52% higher with tripartite inoculation in *A. vasica*. The increase of Fe and Zn in plant tissue due to microbial inoculants especially *Azotobacter* and *Pseudomonas* (PSB) was accentuated (Vansuyt *et al.*, 2007; Indiragandhi *et al.*, 2008) by due to siderophore production by these microbes which mediate the acquisition of aforesaid

nutrients to plant. Several workers have reported that PSB release organic and inorganic acids which change P and other nutrients in available forms ready for uptake by plant (Singh and Kapoor, 1999; Zaidi *et al.*, 2009). In addition, AMF is known for its contribution in nutrient uptake especially P beyond the root zone through extensive mycelium network in mycorrhized plants (Marschner and Dell, 1994; Bhardwaj and Chandra, 2018). Hence, the increment in N, P and other nutrient elements in the plants inoculated with microinoculants resulted to enhance biomass yield. Similar results have been reported by Mahfouz and Sharaf-Eldin, (2007); Chandra, (2015). It has well established that AMF dominantly occupy root and form symbiotic relationship with hosts and play crucial role for partner in terms of higher uptake of nitrogen, phosphorus, zinc, copper and water from the soil (Jiang *et al.*, 2013) and thereby luxurious growth and biomass yield of plants colonized with AMF. *Azotobacter* improves available N into soil through biological N fixation concerning higher N contents in plants (Attia and Saad, 2001; Hassan, 2009) and thereby yields. Thus the combined inoculated *W. somnifera* and *A. vasica* enabled multiple benefits comparing single microbial strain inoculant (Abdullah *et al.*, 2012). Besides better uptake of nutrients plants treated with bioinoculant secrete higher amount of growth hormones such as auxins, gibberlins (Glick, 2012), vitamins and organic acid which increases the surface area of the root length and emergence of higher number of root hair (Mathur and Vyas, 2000; Nandre *et al.*, 2005; Zaki *et al.*, 2010). Similarly the growth promoting traits of *Azotobacter* and PSB has been identified (Ahemad *et al.*, 2008; Wani *et al.*, 2007) depicts the synthesis of

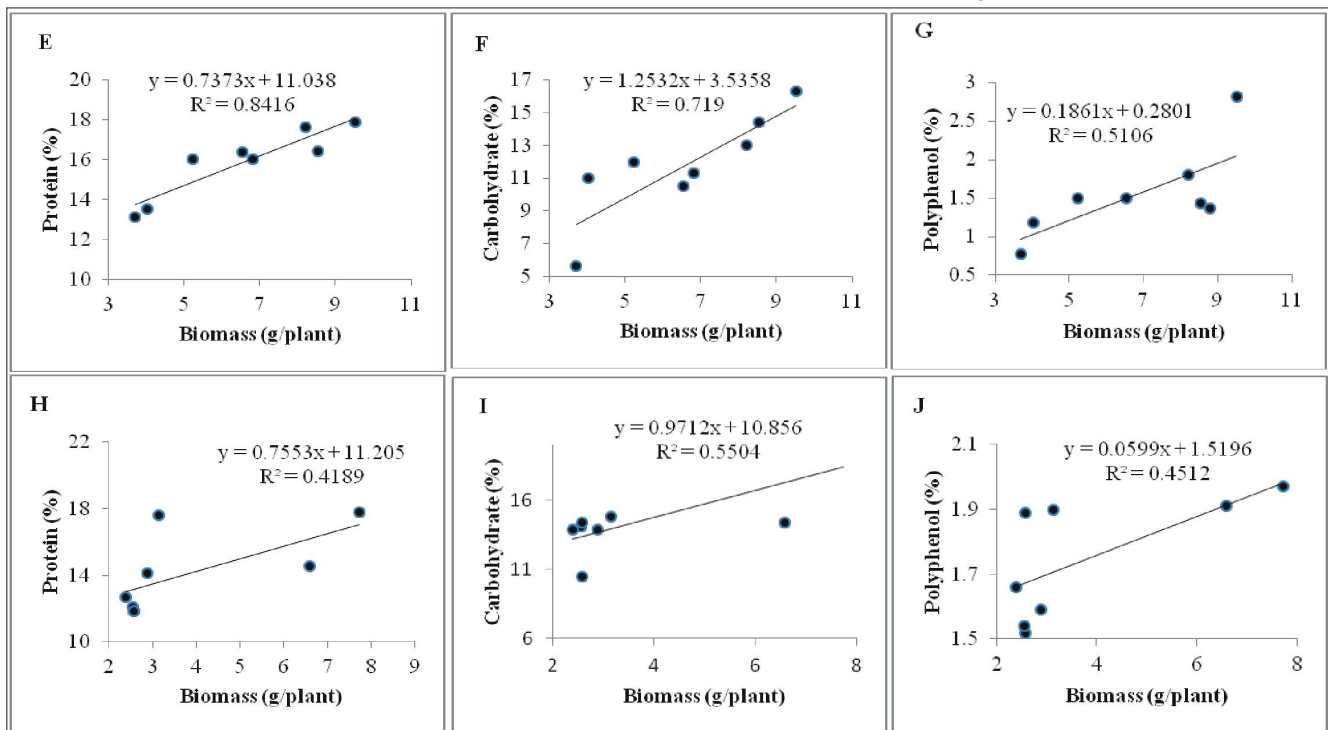
Table 3: Effect of different bioinoculants alone and its combinations on protein, carbohydrate and polyphenol in *W. somnifera* and *A. vasica*.

| Treatment | <i>Withania somnifera</i> | | | <i>Adhatoda vasica</i> | | |
|-----------------------------|---------------------------|------------------|-----------------|------------------------|------------------|-----------------|
| | Protein (%) | Carbohydrate (%) | Poly Phenol (%) | Protein (%) | Carbohydrate (%) | Poly Phenol (%) |
| Control | 13.12 | 8.60 | 0.77 | 11.87 | 10.48 | 1.52 |
| AMF | 16.02 | 12.00 | 1.50 | 12.11 | 14.05 | 1.54 |
| PSB | 16.06 | 11.30 | 1.37 | 12.68 | 13.88 | 1.66 |
| <i>Azotobacter</i> | 13.56 | 11.00 | 1.19 | 11.87 | 14.4 | 1.89 |
| AMF+PSB | 17.87 | 16.30 | 2.82 | 14.12 | 13.9 | 1.59 |
| AMF+ <i>Azotobacter</i> | 16.43 | 14.40 | 1.44 | 17.62 | 14.8 | 1.90 |
| PSB+ <i>Azotobacter</i> | 16.37 | 10.50 | 1.50 | 14.55 | 14.4 | 1.91 |
| AMF+PSB+ <i>Azotobacter</i> | 17.62 | 13.04 | 1.80 | 17.81 | 20.5 | 1.97 |
| CD at $p \geq 0.05$ | 2.55 | 3.73 | 0.20 | 1.99 | 2.67 | 0.31 |

IAA, siderophore and ammonia production, HCN, exopolysaccharides and phosphate solubilizing activities (Ahemad and Khan, 2011a, 2011b; Maa *et al.*, 2011 and Tank and Saraf, 2009). All they mentioned that these functions equip microbes enabling higher growth and biomass yields by making congenial environment in rhizosphere even under adverse condition.

Table 3 shows the effect of bio-inoculants on protein, carbohydrate and polyphenol contents in plants under present investigation. The results were significant in both the medicinal species as the increment was highest 36.20%, 89.53% and 266.23% in protein, carbohydrate and polyphenol respectively in *W. somnifera* inoculated

with AMF+PSB. These attributes enhanced by 50.0%, 95.61% and 29.60% protein, carbohydrate and polyphenol respectively in *A. vasica* with tripartite inoculation. Though other treatments also influence greatly the chemical constituents in both the plant species, however the effect of AMF+PSB and tripartite inoculants was significantly highest in *W. somnifera* and *A. vasica* respectively. Overall, tripartite, dual and single inoculants augmentation ranged 15.92-34.29%, 32.90-59.65%, 75.32 - 133.76% in protein, carbohydrate and polyphenol respectively in *W. somnifera* compared to control. Similarly the increment of these attributes in *A. vasica* ranged 2.94-50.04%, 34.63-59.61%, 11.18-29.60% in protein carbohydrate and polyphenol. The results show in fig. E to J reveals that an inoculation of bio-inoculants significantly increases the biomass and chemical constituents of plants. Consequently, a linear trend with significant effects found between biomass and protein ($r^2 = 0.841$), carbohydrate ($r^2 = 0.719$) and polyphenol ($r^2 = 0.516$) in *W. somnifera* (Fig. E, F and G) while comparatively weak positive correlation found between biomass and protein ($r^2 = 0.323$), carbohydrate ($r^2 = 0.550$) and polyphenol ($r^2 = 0.451$) in *A. vasica* (Fig. H, I and J). The microbial

**Fig.** Correlation Coefficient between biomass Vs (E) protein, (F) Carbohydrate, (G) polyphenol in *W. somnifera* and (H) protein, (I) Carbohydrate, (J) polyphenol in *A. vasica*.

inoculated plants add carbohydrate in its leave and other plants due to improved growth and nutrient uptakes as also reported by Elkeltawi *et al.*, (2003). These results were in agreement with those obtained by Abd El-Raof, (2001). Generally the plant protein increases with the improvement in nitrogen and polyphenol and therefore N increment by bio-inoculants also resulted into plant protein. However, the effectiveness of AMF varies with the plant species which usually depend upon the understanding of both microbe and host for way of mitigating the most limiting factor (Yang *et al.*, 2012; Nouri *et al.* 2014, Ahemad and Kibret, 2014; Alori and Babalola, 2018). Therefore one inoculant may be most effective for a host species may be non-effective for other host.

Conclusion

Experiment result depict that bio-inoculants AMF, phosphorus solubilizing bacteria and *Azotobacter* found beneficial for improving the biomass yield, nutrient uptake and chemical constituents in *W. somnifera* and *A. vasica*. Any increment in biomass yield was directly related to the better nutrition of the plant which was enhanced through the use of bio-inoculant. The combined inoculation of AMF+PSB was found best in *W. somnifera* while tripartite inoculation gave best result in *A. vasica*. Therefore for ecofriendly production technology these bio-inoculants (Biofertilizer's) should be promoted especially in medicinal plants because chemicals and pesticides are prohibited in these crops.

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