International Journal of Botany Studies
ISSN: 2455-541X; Impact Factor: RJIF 5.12
Received: 10-02-2021; Accepted: 06-03-2021: Published: 10-03-2021
www.botanyjournals.com
Volume 6; Issue 2; 2021; Page No. 90-94

Growth of *Triticum aestivum* L and *Sesamum indicum* L. using mycorrhizal root & rhizospheric soil of *Parthenium* as natural vam inoculums

Sudhansu Samanta
Post Graduate Department of Botany, Sabang Sajanikanta Mahavidyalaya, Lutunia, Midnapore, affiliated to Vidyasagar University, West Bengal, India

Abstract
Vesicular-arbuscular mycorrhiza (VAM) symbiosis is a natural phenomenon of any plant community and play unique role in uptake of nutrient and water particularly in nutrient poor soil. Plants infected with VAM fungi can mobilize phosphorus and other nutrients which create a suitable environment for plant survive in nature. The agricultural soil of South West Bengal is low in organic carbon, nitrogen and phosphorus. Use of Vesicular-arbuscular mycorrhiza as bio-fertilizers creates favorable soil ecosystem where phosphorus, nitrogen, potassium and other nutrients get mobilized. In present agricultural practice excess chemical fertilizers, fungicides and pesticide are uses. The objective of the present investigation was to search out the easily available natural mycorrhizal inoculums present in the roots and rhizospheres of the luxuriantly growing weeds of *Parthenium* in this case all around. Plants inoculated with *Parthenium* rhizospheric mycorrhizal soil was proved more efficient inoculums, which could provide more growth compared to root inoculums, in the early age of seedling establishment. It was observed a positive aspect of *Parthenium* plants, whose roots and rhizospheric soil may be used as VAM inoculums for higher growth of *Triticum aestivum* L *and Sesamum indicum* L.

Keywords: *Triticum aestivum* L, *Sesamum indicum L,* rhizospheric soil, *Parthenium*

Introduction

Wheat and sesame are important source of essential components in adequate and balanced human diet. Wheat is important food to human beings. They supply carbohydrates, proteins, fats, minerals, vitamins, antioxidants**.** Cultivation of wheat and sesame has been in practice since humans began to live a settled life. Over the years people have learned how to increase the yield of their crops through breeding and fertilization. In the tropics and subtropics conventional intensive food crops production is characterized by extremely high input of pesticides and fertilizers. The indiscriminate use of synthetic fertilizers and pesticides in production system of food is of great concern for health and environment safety. Research and development strategies are presently diverted in search of suitable biological alternatives to replace the chemical fertilizer and pesticide use. We must go for a combined use of both organic and inorganic fertilizers in judicious combination based on soil nutrient status to improve and sustain the soil fertility and productivity as well as in control of plant diseases in integrated plant yield and disease management which is called as integrated nutrient management (INM). One of the major components in INM practices is the use of bio-fertilizers which are prepared from many beneficial microbial inoculants (Meena *et al*. 2013a, 2016a; Maurya *et al*. 2014; Jat *et al*. 2015; Kumar *et al*. 2015, 2016b; Ahmad *et al*. 2016) [20, 21, 19, 12, 15, 16, 1].
Wheat and sesame when grown in field become mycorrhizal gradually. However because of excess chemical fertilizers and fungicides, pesticides uses the normal microbial population along with the VAM fungi is depleted in the soil which slows down the normal mycorrhization of the field grown crops. Mycorrhizae are an integral part of most plants in nature (Giazninazzi *et al*., 1983) and occur on 83% of dicotyledonous and 79% of monocotyledonous plant investigated (Wilcox, 96). Infection of the root system of the Plant by the mycorrhizal fungi creates a symbiotic (beneficial) relationship between the plant and fungus.
Mycorrhizal benefits have been reported for many cereals, including pearl millet, wheat, and maize (Rao *et al*.1983; Lu and Miller, 1989) [25, 18]. Suri *et al*., in 2006 screened out efficient local VAM strains out of 600 soil samples collected from various crop rhizospheres in wet temperate zone of Himachal Pradesh (India), located in North-Western Himalayas. The crops covered were maize, wheat, oats, berseem, soybean, French bean, onion, potato, garlic, chilies, citrus, apple, pear, peach etc. Maximum spore count (110-185 spores/250 g soil) was recorded under vegetable field soils. It is concluded from above that vegetable field soil may be the best inoculants for mycorrhizal biofertilizer. Sylvia et al. (98) estimated that as much as 20% of the total carbon assimilated by the plant may be allocated to mycrorrhizal fungi.
Upon root infection and colonization, mycorrhizal fungi develop an external mycelium which is a bridge connecting the root with the surrounding soil (Toro *et al*. 1997) [29]. One of the most dramatic effects of infection by mycorrhizal fungi on the host plant is the increase in phosphorus (P) uptake (Koide, 1991) [14] mainly due to the capacity of the mycorrhizal fungi to absorb phosphate from soil and transfer it to the host roots (Asimi, *et al*. 1980) [3]. In addition, mycorrhizal infection results in an increase in the uptake of copper (Lambert, Baker & Cole, 1979; Gildon & Tinker, 1983) [17, 5, 7], zinc (Lambert, Baker & Cole, 1979), nickel (Killham & Firestone, 1983) [13], and chloride and sulphate (Buwalda, Stribley & Tinker, 1983) [5, 7].

Mycorrhizae also are known to reduce problems with pathogens which attack the roots of plants (Gianinazzi-Pearson & Gianinazzi, 1983) [8].

Mycorrhizal advantage to the crops as highlighted above are not available to the crops grown by farmers because cheap and effective source of mycorrhizal inoculums is not in normal chain of supply. This is the major bottleneck towards the application of mycorrhizal inoculums at field level. It was observed repeatedly, year after year that the roots of *Parthenium* show the intensive mycorrhization to the tune of 75% to 100%.

Even the rhizospheric soil of *Parthenium* harbor VAM spores to the extent of 600-700 per 100g soil. As the normal inoculum of mycorrhiza consist of roots and spores, it was thought to test the infectivity of the *Parthenium* roots and its rhizospheric soil as source of mycorrhizal inoculums to grow the wheat and sesame in the plastic pots.

The objective of the present investigation was to search out the easily available natural mycorrhizal inoculums present in the roots and rhizospheres of the luxuriantly growing weeds *Parthenium*.

Materials and Methods

Geographical location: Pot experiment was conducted at the experimental cum botanical garden of Botany Sabang Sajanikanta Mahavidyalaya, Lutunia Pashim Midnapore, West Bengal, India during 2017-2018.Pot experiment was conducted at the experimental cum botanical garden of post graduate department of Botany, Sabang Sajanikanta Mahvidyalaya under Vidyasagar University, west Bengal, India during 2019. It is situated in the Southern region of west Bengal, India, located at 22.2262^0 N latitude and 87.5630^{0}E longitudes at a distance of 86 km from the Bay of Bengal, at elevation of 12m above mean Sea level.

Climate: warm and humid, the average annual rainfall ranges from 1300mm to 1500mm, 80% of which is received during June to October from southwest monsoons.

Soil: The Soil of the experimental pots was sandy loam in texture; It is low organic carbon nitrogen (N), and phosphorus (P) content, and medium in potassium (K) content. The details of physico-chemical characteristic of the soil estimated by standard procedure were followed APHA, 2005.

Table 1: Physico-chemical properties of lateritic soil after Mixing with farm yard manure.

Soil type	pH	Ec mmhos/cm.	Percent Org. carbon	Av. $P_2O_{2.}$ ppm	Av. K_2O.ppm
Prepared pot soil for mung cultivation	6.0	0.06	0.40	50.6	450
Natural soil of experimental garden	5.1	0.05	0.19	40.4	300

VAM inoculum: The inocula used were VAM spores of rhizespheric soil and crushed mycorrhizal roots of *Parthenium* plants. VAM soil inocula were air-dried in shade for two weeks.

Root inoculums: Mycorrhizal root inoculums was properly washed to remove adhering soil. The healthy fine lateral roots were cut into pieces leaving the root tips. The roots were surface sterilized with 0.5% sodium hypochlorite and rinsed with water. The roots were crushed with mortar, pestle, and sieved through 1mm mesh.

VAM spore population of rhizospheric soil of *Parthenium*

Plants: 100gm of air-dried rhizospheric soil was dispersed in 500ml water in a beaker, stirred rigorously and allowed to stand for 30-45 seconds, when the soil settled down. The supernatant was poured through sieves of 200µ, 150µ, 100µ & 50µ mesh. The entire process was repeated thrice. The residues on the sieves were washed with water jet and collected in beaker. The content of the beaker was filtered on whatmon No 1 filter paper and the spores on the filter paper were observed under stereo microscope and the number counted. (Gerdemann & Nicolson, 1963). The total VAM spores per 100g soil varied between600-700.

VAM infection of root

The crushed samples were boiled in 10% KOH in an autoclave at 5lb/m^2 pressure for 10 minutes, washed with distilled water, acidified with dilute HCl, washed with distilled water and stained with 1% trypon blue for 2-3 minutes (Phillips and Hayman 1970) [24]. The Stained root samples were mounted in lacto phenol and observed under compound microscope for VAM infection. The infection percentage ranged between75% to 100%.

Experiments

Pot experiments: Polythene bags of 20cm height and 20cm diameter were used. Fifty-six pots were used for experiments. Pots were arranged in eight rows. The design followed was random block design with 8 replications.

Soil preparation: Soil was collected from the field from top 20cm depth and passing through 2mm size of sieve. The soil was sterilized by1/4th strength formal dehyde(38% formaline) for seven days under polythene cover and opened for 15 days thereafter before use.

Each pot was surface sterilized with formalin and thereafter was filled up with 2kg soil.

Following inocula per pot with 2kg soil were used.

1. 5 gm mycorrhizal root.
2. 10 gm mycorrhizal root.
3. 20 gm mycorrhizal root.
4. 20 gm soil (100 spores).
5. 30 gm soil (150 spores). 6) 40gm Soil (200 spores)

A control without inoculums was kept for comparison.

Wheat and sesame seeds were germinated in natural condition in 35^{0}c and 95% humidity for 3 days before plantation. The VAM inoculums was layered 2-3cm beneath the plants. Watering was done to maintain uniform moisture content in soil in all the pots as and when required. Experiment was conducted between the Month of 4th March to 19th May of 2019.

Observation Records

Sampling procedure: During growing period, leaf samples were collected at 15days, 30 days, 45 days and 75 days after planting (d.a.p). Plant sampling was done at 15, 30, 45 and 75 (d.a.p).For plants sampling, three plants were randomly selected from each treatment.

Growth: The data on plant height, leaf number and leaf area was recorded at 15days, 30 days, 45 days and 75 days after planting. Plants height was considered from ground level to apex of the fully opened apical vegetative bud.

Measurements

The plants height was measured by plain scales. The dry weight was obtained after drying the plants in oven at 80^0c for 48 hours, VAM infection percentage was determined following Phillips and Hayman (1970) [24].

Observation of morphological parameter

For observing and recording different morphological parameters five plants were randomly selected from all treatments.

All under mentioned parameters were recorded at at 15days, 30 days, 45 days and 75 days after planting except the fruit and plant dry weight. Plant and fruit dry weight was recorded after 75 d.a.p. VAM infection percentage was also observed after 75 days.

1. Plant height
2. No of leaf
3. Leaf area.
4. Plant dry weight
5. Fruit dry weight
6. VAM infection percentage

Result

Table 2: Table showing Plant height, leaf number and leaf area of Wheat plants (*Triticum aestivum L.*) under different VAM inoculums treatments in 15,30,45,75 days after planting (D.A.P).

Treatment	Plant height (cm)				Leaf number /plant				Leaf area / plant (cm^2)			
	15 D.A.P	30 D.A.P	45 D.A.P	75 D.A.P	15 D.A.P	30 D.A.P	45 D.A.P	75 D.A.P	15 D.A.P	30 D.A.P	45 D.A.P	75 D.A.P
5gm root	2.15	4.47	9.57	15.1	2.4	3.8	4.3	4.7	43.16	83.19	86.28	98.33
10gms root	3.09	4.6	10.9	14.7	2.6	3.8	3.6	4.4	59.19	79.94	97.01	72.39
20gm root	3.42	5.36	9.35	13.4	2.2	3.6	3.8	4.4	55.1	104.3	133.25	109.2
20gm soils	4.4	6.25	14.6	17.4	2.6	3.2	3.4	4.8	96.57	119.4	183.34	74.9
30gm soils	5.27	3.45	13.9	18.8	2.8	3	4	4.6	96.12	135.5	183.36	60.74
40gm soils	4.24	5.86	10.9	19.9	2.4	3.2	3.6	4.4	57.93	153.9	153.89	63.14
Control	4.64	5.98	13.9	15.6	2.6	4	4	4.2	73.68	130.5	130.48	79.92

Note: all results in three replicates.

Highest plant height 19.9cm was observed in mycorrhizal spores with 40gm soils inoculum followed by 30gm and 20gm mycorrhizal spore inoculum but in 20gm mycorrhizal root treatements showed least plant height than 10g and 5gm mycorrhizal root inoculums. There were no significant changes in leaf numbers among treatments in wheat plants.In case of leaf area in wheat plants, mycorrhizal soil inoculum promote higher leaf area than control but mycorrhizal root treatments showed lower leaf area than control (Table-1). All rising trends in case of leaf area were observed up to 45 D.A.P. and showed sharp decline at 75 D.A.P.

Table 3: Table showing Plant dry weight, fruit dry weight and VAM infection percentage of Wheat plants (*Triticum aestivum L.*) under different VAM inoculums treatments in 75 days after planting (d.a.p).

Treatment	Plant dry weight (mg)	Fruit dry weight (mg)	VAM infection percentage
5gm root	1332	460	50
10gms root	1106	470	60
20gm root	864	920	60
20gm soils	1106	600	60
30gm soils	1077	620	60
40gm soils	1094	2580	90
control	1042	133	0

Plant dry weight were observed higher in case of 5gm root treatments and lowest in case of 20 gm root treatments but in all other cases no significant difference was observed. Highest yield of wheat 2580mg grain were harvested in 40gm mycorrhizal soil inoculums followed by 920mg grain yield in 20gm root treatment. .In mycorrhizal treatments wheat grain yield were 3 to 19 times higher than non-mycorrhizal control. Highest VAM infection % (90%) were observed in 40gm soil inoculums treatments and lowest in 5gm root inoculums, in other treatments showed no differences in VAM infection %. (Table-2)

Table 4: Table showing Plant height, leaf number and leaf area of Sesame (sesamum *indicum L.*) under different VAM inoculums treatments in 15,30,45,75 days after planting (D.A.P.).

Treatment	Plant height (cm)				Leaf number /plant				Leaf area / plant (cm^2)			
	15 D.A.P.	30 D.A.P.	45 D.A.P.	75 D.A.P.	15 D.A.P.	30 D.A.P	45 D.A.P	75 D.A.P.	15 D.A.P.	30 D.A.P.	45 D.A.P.	75 D.A.P.
5gm root	5.6	7.65	12	23.7	3.6	4	5.8	11	14.36	20.3	73.97	504.7
10gms root	4.57	7.22	10	21.8	3.8	3.8	6	10	12.76	19.34	69.65	309.8

20gm root	4.24	7.71	11.9	21.4	3	4	5.6	9.6	9.77	18.19	74.43	347.3
20gm soils	5.04	6.61	10.3	20.8	3	3.6	5.8	10	11.23	12.58	64.62	303.5
30gm soils	6.08	7.15	9.58	20.6	3.4	3.8	5	11	10.96	14.12	54.8	420.6
40gm soils	5.86	7.87	11	20.6	3.6	4	6	12	12.76	16.66	69.17	574
control	6.44	5.78	9.36	19.3	4	3.8	5.2	9	16.05	15.9	57.8	307.8

In Sesame plants all the mycorrhizal treatments showed higher plant height than control plant (Table-3). Mycorrhizal treatments showed higher leaf number same as plant height except 30gmsoil treatment where leaf number was lower than control. Mycorrhizal treatments promoted higher leaf area than control and highest 574 cm^2 in 40gm soil treatments. (Table-3).

Table 5: Table showing Plant dry weight, fruit dry weight and VAM infection percentage of Sesame plants (Sesamum *indicum L.*) under different VAM inoculums treatments in 75 days after planting (d.a.p).

Treatment	Plant dry weight (mg)	Fruit dry weight (mg)	VAM infection percentage
5gm root	1362	No fruit	50
10gms root	1813	310	50
20gm root	1962	368	70
20gm soils	1214	180	40
30gm soils	1488	340	50
40gm soils	1872	450	90
control	1334	No fruit	3

1962 mg plant dry weight was observed in 20gm root treatments followed by 1872mg in 40gm soil inoculums, 1813mg in 10gm root, 1488mg in 30gm soil, 1362mg in 5gm root and 1334mg in control.

There was no fruit yield of sesame in 75 days after plantation in both control and 5gm mycorrhizal root treatments. Maximum fruit yield 450mg recorded in 40gm mycorrhizal soil treatments. Treatments with higher spore number or root biomass encouraged more fruit yield in sesame (Table-4).

Discussion

The soil of Paschim Medinipur, West Bengal, India has low pH and low soil moisture. It is deficient in available phosphate and other nutrients and to a certain extent in nitrogen and organic carbon as well. (Jana and Das, 1987 and Sharma *et al*, 1990). The acidity factor and low moisture level tend to immobilize the phosphorus as bound iron and Aluminum phosphates and thus reduce its availability (Mondal and Mondal, 1990). Besides this, leaching of nutrient is a natural process due to high porosity and light weight of the soil. All these factors reduce the availability of nitrogen, phosphate, zinc and other micro and macro nutrients. (Jana and Das, 1987, Dutta *et al*., 1989) [11]. In such a soil, addition of chemical phosphate fertilizer is also of little use, since they too are fixed, become immobile and unavailable. The red lateritic soil creates nutrient and moisture stress to the plant growth. VAM facilitate better survival of plants under stress condition through a boost in uptake of nutrients particularly P, Zn, Cu & water (Graham *et al* 1971, Auge *et al*, 1986) [4]. Nelson and Safir (1982) [23] observed the increased drought tolerance of mycorrhizal onion plants caused by improved phosphorus nutrition. Plants inoculated with *Parthenium* rhizospheric mycorrhizal soil was proved more efficient inoculums, which could provide more growth compared to root inoculum, in the early age of seedling establishment. All the observation suggested that soil inoculum helped to produce plant growth in early stage which continued to be same at latter stage of plant growth also. Soil inoculum of 200 spores also proved as efficient as 20 gm root inoculum for the growth of the plants. Therefore, it was clear that soil inoculum had more potential for growth enhancement. After 75 days of plantation of sesame plants there were no fruit initiation in control and 5gm root inoculums but all other mycorrhizal treatments promoted fruit growth clearly suggesting that mycorrhizal association are very essential for plant growth as well as for higher fruit yield. Although *Parthenium* is considered a major problem (Gupta and Sharma 1977; Shelke 1984) [10, 27]; and Parthenium is one of the feared weed species (Rao, 1956), It was observed a positive aspect of *Parthenium* plants, whose roots and rhizospheric soil may be used as VAM inoculum for higher productivity of agricultural crops and trees. Once this result is repeated at field level crop production with other hosts, the parthenium will find a separate identity and the eradication of the weed would be a thing of past.

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