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# Commonly used pesticides in agriculture of Warangal District-Telangana and their consequences on the growth of Vesicular and Arbuscular Mycorrhizal fungi with reference to the DNA and Protein Content

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***Abstract:** In this paper we described the hazardous affects of the four common pesticides Captain, Bavestin, Endosulfan, Dicofol on VAM growth with reference to DNA ratio and protein content. Initially, we isolated VAM spores from farm lands and inoculated in to sterilized soil that contain selected plant seeds. The tests were performed to isolate the VAM and to determine the DNA ratio and protein quantity. The highest number of VAM spores are noticed from BAV-I sample pot are 187. The DNA ratio at 260/280 was found significant with VAM isolated from DIC-I 72ng. In our investigation we identified two different genus of VAM are Glomus and Gigaspora. The highest protein content 82.6 was recorded with VAM samples of CAP-I and II.*
***Keyword:** Glomus , Gigaspora Captain, Bavestin, Endosulfan, Dicofol*

## I. INTRODUCTION

Interaction of pesticides with living cells is a common process. The extensive use even than the above safer limits will leads to adverse relationships. This type of adverse relationship is most widely studied using VAM fungi. Being emerging model system, vesicular arbuscular mycorrhiza (VAM), scientists are now focused on crop improvement and development. Generally, crop plants benefited from the VAM infection and association by greater absorption of phosphorus and water from soil [1]. Scientists reported that the infection and colonization of VAM minimize drought resistance in wheat plants [2-3]. The current work was framed to evaluate the hazardous effects of pesticides on VA-mycorrhizal growth in concern to its DNA ratio and protein quantification. The AM fungi are the commonly studied variety of mycorrhizae due its abundant presence in agricultural and natural ecosystems in which that play importance role in plant growth and development.

Now-a-days, agricultural farmers are extensively in use of fungicides in larger quantities order to control and kill fungal phytopathogens. However, fungicides applied on soils not only affect phytopathogens but also are capable of showing side effects on autochthonous soil microorganisms that are actively involved in development of plant [4-7]. The beneficial effects of VAM fungi on the plant growth and products can be hampered by the wide use of pesticides in agricultural systems [8]. Several studies documented that the length of external hyphal of VAM has been decreased by the higher levels of fungicide treatments with the subsequent reduction of phosphorus (P) content in plants [9].

In the context to the effect of pesticides on VAM the present study was framed to evaluate the hazardous affects of commonly used pesticides in agricultural fields of Warangal district.

## II. MATERIAL AND METHODS

Two sampling sites were selected for my research work:

Farm lands on (surrounding Warangal city).

Farm lands (own farm land (Pochampalley), Nancharimadur, Kodakandla road, Warangal District. Location: 32.69°N 71.12°E Altitude: 412 m (1742 ft.) Above Sea Level Climate: Hot Semi-Arid Type Summer temperature: Minimum: 25° C, Maximum: 45°

C.

*A. Isolation Of Vam Spores From Rhizosphere Soil Mixtures*

Spores were isolated from field-collected root-rhizosphere soil mixtures. Spores of arbuscular fungi were isolated by using the 'wet sieving and decanting method' described by Gerdemann and Nicolson, 1963 [10].

The following steps were made: In soil remove the coarse materials like straw, debris and rocks should be removed with a 2-mm sieve.

*1) Soil Sample Mixed With Tap Water*

*a)* 100 gm of air-dried root-rhizosphere soil mixture were placed into a glass container with 1000 ml of tap water.

*b)* The root-soil mixture was vigorously mixed with a glass rod for 30 seconds. Figure 2: Soil suspension sieved

*c)* A 10-second pause enabled to settle heavier particles and organic material, the remaining soil-water suspension were slowly poured through a set of two sieves. The sieves used are those with pores of diameters of 0.5mm (the top one) and 0.045 mm (lowest one). Most spores retain on the 0.045 mm sieve.

*d)* The extracts were washed away and spores collected from the sieves in to Petri dishes.

*e)* Using a microscope, spores and aggregates were picked by means of dropper and needle. Figure 4: Isolated spore picked with dropper under microscope

*f)* Selected spores were separated with a needle. A drop or two of mountant (poly vinyl lacto glycerol) was spread on the centre of a clean and dry slide so as to hold cover slip. Spores were placed on the mountant and the cover slip was placed gently by avoiding air bubbles. Such prepared slides were labeled, allowed to dry in a dust free chamber for 3-5 days. The edge of the cover slip was sealed with clear nail polish to prevent the desiccation and entry of air bubbles and Spores were examined.

*B. Fungal Spore Inoculation*

The isolated VAM fungal spores are inoculated in to the pots containing sterilized soil along with the various pesticides that are commonly used in the farm lands in Warangal District. The pots were then filled with paddy seeds of for the growth and development. The pots were categorized in to four groups and labeled the pots according to the pesticide that inoculated in to the soil. The following groups are made;

Gropup-I: VAM spores inoculated with Captain pesticide 100 mg/L
Gropup-II: VAM spores inoculated with Bavestin pesticide 100 mg/L
Gropup-III: VAM spores inoculated with Endosulfan pesticide 100 mg/L
Gropup-IV: VAM spores inoculated with Dicofol pesticide 100 mg/L

*C. Isolation and Identification Of Mycorrhizal Spores*

Isolation of mycorrhizal spores was carried out by wet screening methods and methods of sucrose gradient centrifugation [11]. The wet screening technique was conducted by weighing 100 g soil samples and the dissolved in 1000 ml of water then left for 10-15 minutes to allow the sediment to settle. The suspension then filtered by pouring it into filter with diameter of 40 µm, 50 µm, and 200 µm, respectively, repeated 3 times. Spores were filtered through a sieve of 50 µm and 200 µm then inserted into the centrifuge tube. A 2500 rpm speed was used to centrifuge the suspension for 5 minutes. Supernatants were collected on the top in the exhaust up to three-quarters of the tube. The remaining solution in the tube was mixed with 60% sucrose solution then centrifuged at 1200 rpm for 2 minutes. The suspension is poured into 10 cm diameter petri dishes. Further, the suspension between the water and the sugar was taken and placed on a 200 µm sieve, sieve sprayed with water slowly to clean up spores from the remnants of attached sugar. Clean spores were placed on a petri dish diameter of 10 cm to be observed and counted under a dissecting microscope. Subsequently, population of each of mycorrhizal fungi types was counted for 100 g soil then separated on a petri dish based on shape, color and size. Based on these observations, type, population, form and spore color of the mycorrhizae were matched with standard spores [12-13]. Mounting procedure was conducted as follow left hand side of a glass object was dropped into a solution of PVLG and a portion of Melzer solution was dropped onto the right hand side. Similar spores were placed on each of the solution then each surface was covered with a cover slip. Spores were crushed by pressing the cover slip surface with a toothpick [14]. Mixtures of mycorrhizal spores were observed under compound microscope equipped with digital camera with enlargement of 100-400 times.

The identification based on morphological characteristics of spores that is based on size, color, cell walls layer, ornaments, and hyphae form attached to the spores cell walls (bulbous suspensor, hyphae holder, or subtending hyphae). Color change of spores in Melzer solution is one of the indicators to determine the type of spore.

*D. Isolation of Vesicular and Arbuscular Mycorrhiza Genomic DNA*

The genomic DNA of VAM was extracted from five to seven days old fungal cultures grown either in liquid broth medium. The fungal mass from the culture broth was obtained by filtering the culture broth through a 10 ml syringes containing glass wool that will allow the broth to pass through, while retaining the fungal mass. The fungal mass obtained was placed in a 2ml tube containing a ceramic pestle, 60–80 mg sterile glass beads (425–600 µM, Sigma) and lysis buffer (100 mM Tris HCl [pH8.0], 50mM EDTA, 3% SDS). Homogenization of fungal mass was done twice in a FastPrep®-24 tissue homogenizer (MP Biomedicals, USA) at 6 M/S for 60 sec. The resulting fungal tissue homogenate was centrifuge at 13,000 rpm for 10 min and supernatant was transferred to a fresh microcentrifuge tube. To the supernatant, 2 of RNase A (10mg/ml) was added and incubated at 37°C for 15 min. After the RNase A treatment, equal volume of phenol: chloroform: Isoamyl alcohol (25:24:1) was added and mixed well, followed by centrifugation at 13,000 rpm for 10 min (Note: this step can be repeated once more to completely get rid of proteins/cell debris). The upper aqueous layer was taken in a fresh micro centrifuge tube and then equal volume of 100% ethanol was added. Following precipitation at -20°C for 30 min, the whole content was centrifuged at 12,000 rpm for 10 min to pellet down the DNA. The DNA pellet was washed with 70% ethanol and centrifuged at 12,000 rpm for 5 min. The DNA pellets were air dried and dissolved in 1× TE buffer.

*E. Quantity And Quality Determination Of DNA*

The quantity of the extracted DNA was determined by measuring the absorbance at 260 nm using Thermo Scientific Nano Drop 1000 spectrophotometer. All the DNA isolated samples were sent to Centre for Cellular and Molecular Biology (CCMB), Hyderabad to evaluate the purity of the DNA isolated from the VAM fungi by Nano Drop Technology.

*F. Protein Extraction From Vam Cultures*

*1) Organic Medium (OM):* Media used for the extraction of protein from the VAM cultures comprises of 1% glucose (10 g/L), 0.1% Peptone (1g/L), 0.01% Yeast Extract (0.1g/L), 0.1% KH2PO4 (1 g/L), 0.03% MgSO4 7H20 (0.3g/L) or MgSO4 anhyd. (0.146 g/L). All the chemicals are analytical grade and purchased from SD fine chemical Laboratories, Mumbai, India.

*2) Keeping spores in Silica-Gel:* Sterilize silica gel in small capped glass vials in 180$^{o}$ C oven for 1 day, Sterilize 5% (w/v in $H_2O$) powdered milk (autoclave), Add approx. 4ml of milk to each petrie-plate containing sporulating fungal culture. If there is a lot of mycelium, filter the suspension through gauze. Add 0.2ml of spore suspension to each vial containing silica gel. Let air-dry for one week in vials with loosely screwed caps, Tighten caps for permanent storage. To revive culture, inoculate plates with 3-4 pieces of silica gel Protein Isolation from Frozen Ground Tissue Protein Isolation Buffer (PIB) 10 mM Tris-HCl pH 8.0, 1mM EDTA, 2% PVPP. Shake buffer before use. Add proteases inhibitors (50ul each in 10ml): chymostatin, aprotinin, leupeptin plus 500 ul PMSF in methanol. Use 5ml of PIB per 2 g frozen tissue. Pour over frozen tissue and allow it to thaw on ice. Centrifuge at 8000 rpm for 30 min at 4$^{o}$ C. Collect supernatant and add equal volume of acetone (freeze O/N). Centrifuge for 30 min at 6000-7000 rpm (4$^{o}$ C). Pour off supernatant and allow pellet to air dry. Resuspend pellet in 1 ml TE (10 mM Tris-HCl, 1mM EDTA, and PMSF) (crude extract).

## III. RESULT AND DISCUSSION

*A. Isolated of Vesicular Arbuscular Mycorrhiza Fungi*

The current study we have isolated highest number of VAM spores 182 was found in the area grouped as BAV-I followed by CAP-I and BAV-II with 168 and 167 VAM spores respectively of farm lands (I). The results were represented in the table 1.

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Table 1. Enumeration of Vesicular Arbuscular Mycorrhiza Fungi per 100 gm of soil samples

| Samples | | | Number of VAM spore population per 100 gm of soil Collected from the pots inoculated with various pesticides | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Sample-1 | Sample-2 | Sample-3 | Sample-4 | Sample-5 | Total | Type of VAM |
| Study site | Farm lands (I) | BAV-I | 33 | 30 | 52 | 39 | 28 | 182 | *Glomus sps, Gigaspora* |
| | | BAV-II | 25 | 36 | 36 | 45 | 25 | 167 | *Glomus sps, Gigaspora* |
| | | CAP-I | 48 | 43 | 36 | 21 | 20 | 168 | *Glomus sps,* |
| | | CAP-II | 40 | 36 | 30 | 26 | 25 | 157 | *Gigaspora, Acaulospora* |
| | Farm lands (II) | END-I | 23 | 30 | 47 | 32 | 30 | 162 | *Glomus sps,* |
| | | END-II | 29 | 24 | 33 | 39 | 23 | 148 | *Glomus sps, Gigaspora* |
| | | DIC-I | 37 | 29 | 22 | 18 | 28 | 134 | *Glomus sps, Gigaspora* |
| | | DIC-II | 25 | 25 | 18 | 24 | 20 | 112 | *Glomus sps, Gigaspora* |

CAP-Captain, BAV- Bavestin, END- Endosulfan, DIC- Dicofol

*B. Isolation And Identification Of Micorrhiza*

The identification of VAM spores from the soil samples collected from rhizosphere layer was form farm lands I and IIrevealed different types of VAM. Microscopic examination was performed to observe and determine the genus of VAM spores from the soil. Based on the data of our investigation data we identified two different genus of VAM they are i.e. Glomus, Gigaspora.

*C. Isolation Of Vesicular And Arbuscular Mycorrhiza Genomic DNA*

The fungal DNA extraction method yielded high quality molecular weight DNA from the fungal samples collected. The total genomic DNA yield was ranged DIC-I followed by DIC-II (See table 2). Other soil samples collected from farm lands (I and II) showed low DNA 260/280 ratio.

Table 2. Purity of DNA 260/280 ration per 200 mg of VAM fungi samples collected from different regions of Warangal

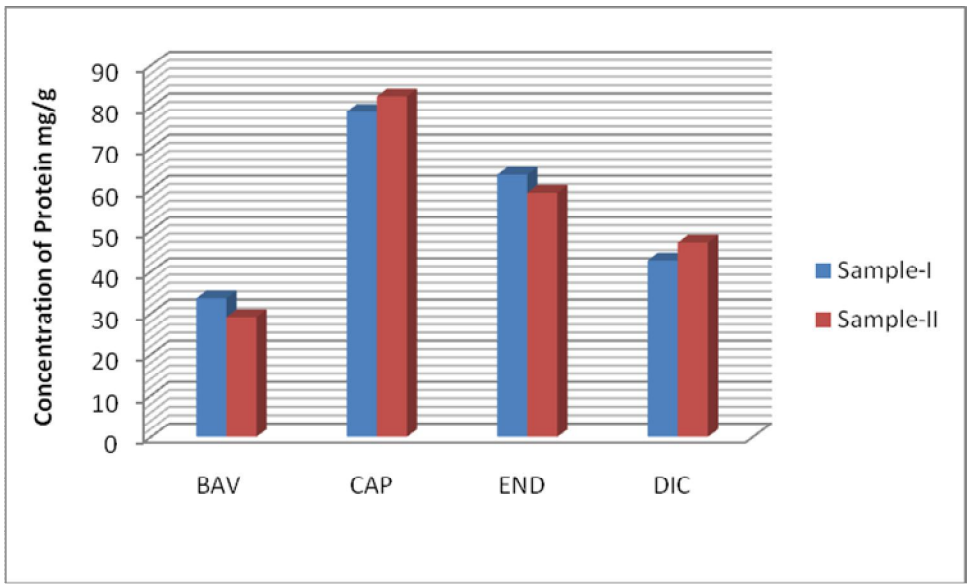
| S. No | Sample | 260/280 | Ratio | DNA conc. |
|---|---|---|---|---|
| 1 | BAV-I | 19.45 | ~1.8 | 36ng |
| 2 | BAV-II | 15.56 | 0.5 | 25ng |
| 3 | CAP-I | 16.13 | 0.8 | 31ng |
| 4 | CAP-II | 18.30 | 1.6 | 40ng |
| 5 | END-I | 22.01 | 2.0 | 49ng |
| 6 | END-II | 18.22 | 1.5 | 41ng |
| 7 | DIC-I | 23.89 | 2.3 | 72ng |
| 8 | DIC-II | 21.01 | 2.1 | 61ng |

CAP-Captain, BAV- Bavestin, END- Endosulfan, DIC- Dicofol

*D. Estimation Of Protein Isolated From Vam Cultures*

The study was designed to determine the protein content in the VAM samples isolated from soil samples collected from different

farm lands. According to our study, the protein content of VAM present in was found high 78.9 and 82.6 in CAP-I and II samples. The protein content of VAM culture samples cultured using different pesticides showed low protein content. The results are represented graph 1.




Graph 1. Estimation of protein using Lowrry method from the VAM samples isolated from the farm lands of Warangal city and Pochampalley village, Warangal District

## IV. CONCLUSION

The present study concludes that Bavestin exhibited the hazardous effects on VAM growth and directly influence the concentration of DNA and Protein. In future we have decided to investigate the effects of some other pesticides which are common use by farmers in agriculture.

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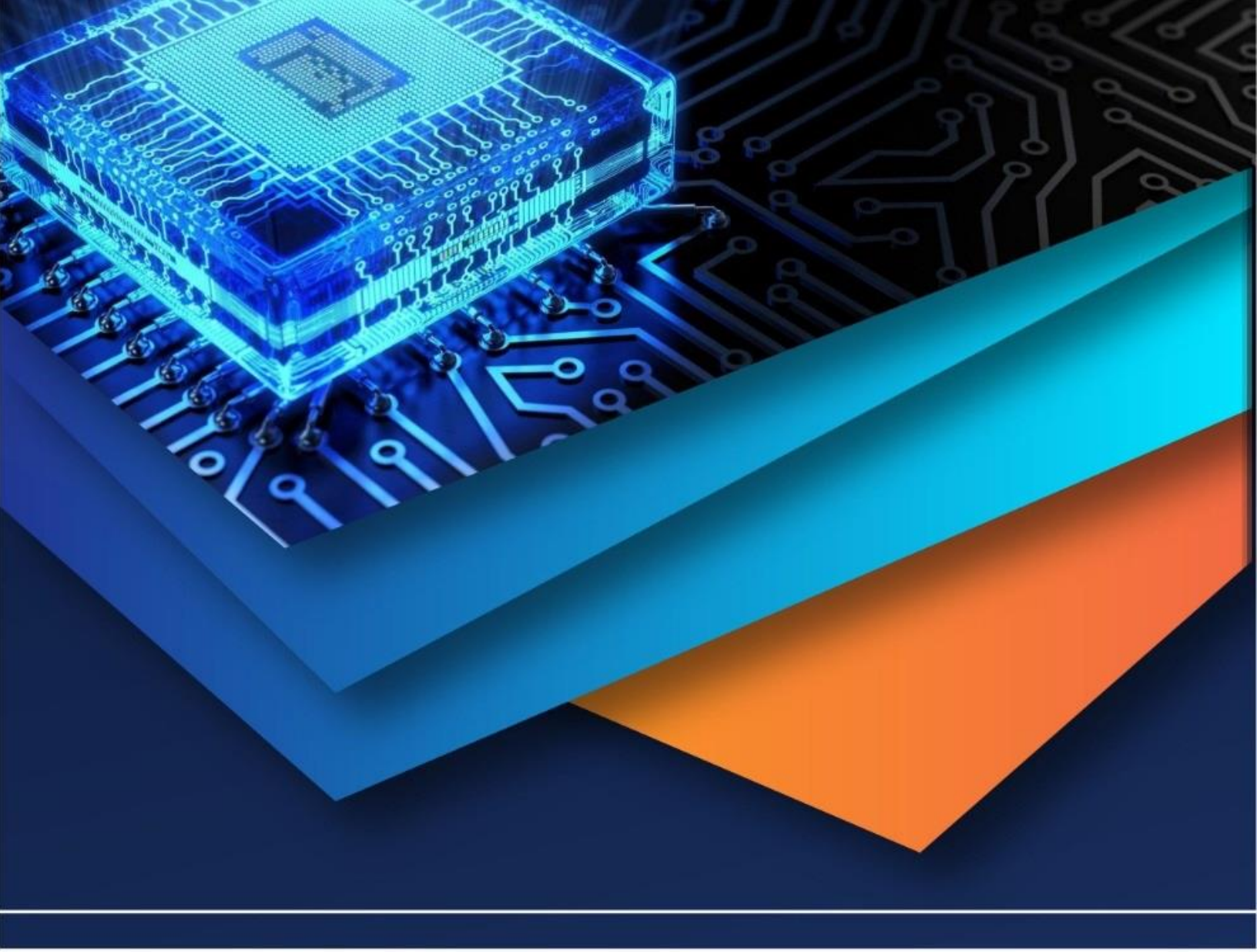

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