

To Study The Effect of Pesticide Resistant *Azotobacter* spp. For The Production of Biofertilizer

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Abstract:

Introduction:

India is an agricultural based country. For fulfilling growing demand of growing population, there is a need to increase agricultural yield. In the era of green revolution chemical fertilizers are successful to increase the agricultural yield but they show some adverse side effects like soil pollution, water pollution and environment pollution.

Low and unbalanced chemical fertilization without organic sources has lead to improper mineralization of nutrients resulting in reduction in crop productivity and low quality of the produce which will fetch low price in the market. It has now become an imperative task to restore the soil with a beneficial microbe population by using bio-fertilizers. From last decade, the bio-fertilizers are able to replace chemical fertilizers and reduce their consumption. In return, micro-organisms provide nutrients for the plant.

Plant Growth Promoting Mechanisms By *Azotobacter*:

Nitrogen Fixation:-

Nitrogen fixation is one of the most beneficial processes performed by *Azotobacter* and other rhizobacteria. Nitrogen is a vital nutrient to plant growth. There is about 78% nitrogen in the atmosphere, but it is unavailable to growing plant. Gaseous nitrogen (N₂) is not available to them due to high energy required to break the triple bonds in two molecules. *Azotobacter* through nitrogen fixation is able to convert gaseous nitrogen (N₂) into ammonia (NH₃) making it an available nutrient to the host plant which can support and enhance the plant growth.

Siderophore Production:-

Micro-organisms and plants require a high level of iron. Obtaining sufficient iron is even more problematic in the rhizosphere where plant, bacteria and fungi compete for iron. Iron is a vital nutrient for almost all forms of life to survive. *Azotobacter* species are known to enhance the plant growth by producing siderophore as iron scavenger and its ability to control the growth of phytopathogenic fungi.

Review of Literature:

Excess and indiscriminate use of chemical fertilizers and pesticides has deteriorated the soil health thus impairing the fertility status of soil, affecting porosity and water holding capacity. Such soil becomes unfit for crop cultivation. In addition to this in recent years there is fertilizer scarcity and escalation in their prices which has become unaffordable to farmers for their usage. In this context there is need to develop low cost, eco-friendly agriculture technologies.

In nature, certain microorganisms have the capacity to mobilize plant nutrients and helps in partial substitution of chemical fertilizers. The literature pertaining to the response of bio-fertilizers on growth and yield of crop plants have been reviewed and presented in this chapter.

Materials and Methods:

Isolation of Pesticide Resistant Bacteria:

Collection of Sample:-

For isolation of pesticide resistant bacteria, soil sample was collected from agricultural field at Hiware Bazar, Ahmednagar, Maharashtra, India, which was highly exposed to DIMETHOATE 30% EC pesticide and NPK Fertilizers. Sample was collected from rhizosphere area in the September and stored in sterile vials.

For isolation, 1gm of soil was serially diluted and 0.1 ml sample from 10⁻⁶ dilution tube was inoculated separately in each of 50 ml of sterile Ashby's broth containing different concentration of pesticide - 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0 % v/v DIMETHOATE 30% EC pesticide.

Characterization and Identification of Isolate:

The isolate was identified on the basis of its morphological, cultural and bio-chemical characteristics. The morphological characteristics of the isolates studied included cell shape, arrangement of cells, pigmentation, Gram nature etc. The isolate was further subjected to the bio-chemical characterization for identification of organism. The bio-chemical tests performed were Catalase, Oxidase, H₂S production, Urease test, Sugar fermentation like Glucose, Sucrose, Lactose, Maltose etc.

Results:

Isolation of Pesticide Resistant Bacteria from Soil Sample:-

After 72hrs incubation, on sterile Ashby's agar plates containing 0.5,1.0,1.5,2.0,2.5,3.0, 3.5, 4.0, 4.5, 5.0% v/v DIMETHOATE 30% EC pesticide, only single type of colonies was observed up to 3% pesticide concentration, which was further purified and used for further study.

Characterization and Identification of Isolate:-

After 72hrs incubation on sterile Ashby's agar plate containing 0.5, 1.0, 1.5, 2.0, 2.5, 3.0% v/v Dimethoate 30% EC pesticide, following characters were observed for the isolate.

Table No. 1:- Summary of biochemical tests

| Sr. No. | Biochemical Test | Result |
|---------|----------------------|---------------------------|
| 1. | Catalase | Positive |
| 2. | Oxidase | Positive |
| 3. | Glucose fermentation | Positive + gas production |
| 4. | Sucrose fermentation | Positive + gas production |
| 5. | Lactose fermentation | Positive + gas production |
| 6. | Maltose fermentation | Positive + gas production |
| 7. | Hydrogen test | sulphide |
| 8. | Urease test | Positive |

Table No. 2:- Antifungal activity

| Sr. No | Fungal species | Diameter of zone of inhibition (mm) |
|--------|----------------------|-------------------------------------|
| 1 | Aspergillus Niger | 23 |
| 2 | Aspergillus flavous | 32 |
| 3 | Alternaria alternate | 29 |

Discussion:

Bio-fertilizers are able to replace chemical fertilizers to increase the yield of crops. Tremendous research is going to isolate different types of bio-fertilizers. But such bio-fertilizers have many limitations like their susceptibility to pesticides, heavy metals, fluctuating physiological condition etc. So, now a day's researchers are trying to isolate bio-fertilizers which are able to resist these conditions. Current work is focused on production of pesticide and salt resistant bio-fertilizers.

Conclusion:

Chemical pesticides may affect plant growth promoting micro-organisms, and inhibit their plant growth promoting ability. It is reported by many researchers that salt concentration of soil increases due to many reasons, the high salt concentration also affect the micro-organism present n soil. To overcome this problem, the current work was focused on production of pesticide resistant nitrogen fixing bio-fertilizer.

Therefore it is evident that the isolated Azotobacter spp. was able to increase the growth of plants and hence can be used as Bio-fertilizer in the fields exposed to pesticide