Methyl red test:

Positive methyl red test

negative methyl red test

Voges-proskauer test:

Positive V. P. test

negative V. P. test

Citrate utilization test

Positive citrate utilization test negative citrate utilization test

Fig2: Biochemical characterization of isolate

Azotobacter is gram negative bacteria and all positive biochemical tests whereas rhizobium is also gram negative in nature and all negative biochemical tests is observed.

Pesticide tolerance:

Plant growth promoting rhizobacteria showed significance tolerance to pesticides studied, indicating their ability to survive under high pesticide stress conditions.

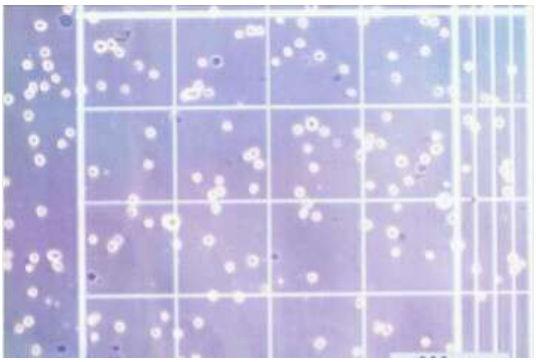
The pesticide had a negative impact on growth of most isolate. Azotobacter isolates showed high optical density at high pesticide level as compare to rhizobium isolate and their growth decreased with increasing concentration of pesticide in case of rhozobium.

Strain	Control	100mg/ml	250mg/ml
Azotobacter	0.63	0.65	0.66
Rhizobium	0.58	0.62	0.61

Table

Viability of isolate:

Live cell count+ dead cell count/Live cell count = %viability



Azatobcter (250mg/L)

The viable cell count for each of the 16 squares were 45, 50, 52, 55

The dead cell count for each of the 16 square were 12, 15, 14, 17

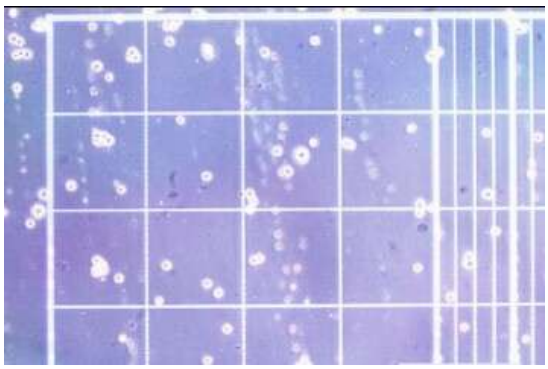
Average viable cell count= 45+50+52+55/4 = 50.5

No of viable cells/ml = $50.5\times10^4\times1$ = 5, 05000 live cells/ml

Dead cell count= 12+13+11+10/4 = 11.5

No of dead cell/ml = $11.5\times10^4\times1$ = 115000 dead cells/ ml

% of viability= 505000+115000/505000× 100 = 81.45%



Rhizobium (250mg/L)

The viable cell count for each of the 16 squares were 18, 15, 17, 19

The dead cell count for each of the 16 square were 8, 10, 7, 8

Average viable cell count = 18+15+17+19 /4 = 17.25

No of viable cells/ml = $17.25\times10^4\times1$ = 172500

Dead cell count = 8+10+7+8/4 = 8.25

No of dead cells/ml = $8.25\times10^4\times1$ = 82500

% of viability = 172500+82500/172500×100 = 52.2%

Pesticide degradation assay:

This assay shows the zone of inhibition around the well. The different concentration of pesticide is added in different well.

The better result is obtained when the mixture of azatobacter and rhizobium suspension were added in the well and the zone of inhibition is clear and maximum

Growth at different pH level:

Rhizobium show maximum optical density at neutral pH. The optical density was decreased with decreasing pH level from neutral to acidic as shown in TABLE 4.

Strain	5	7	9
Azatobacter	0.21	0.45	0.41
Rhizobium	0.24	0.44	0.42

Summary and conclusion:

PGPR are known for their plant growth promotion capabilities. We have checked the capacity of agriculture field isolates to tolerate pesticide compounds. The result of present study show the isolated PGPR strains are capable to degrade the different concentration of pesticide and use it as carbon source and energy.Azatobacter and rhizobium isolate showed significance tolerance, indicating their ability to survive under high pesticide stress condition. In this study Azatobacter show better zone of inhibition as compare to rhizobium isolate but when the culture of both are mixed and used for the degradation the clear zone of inhibition is observed.Azotobacter is more tolerant to pesticide as compare to rhizobium isolate they use the pesticide as carbon source. Morphological characteristics vary in both the isolate that is shape, texture, elevation, opacity, etc.The bacterial isolate were characterized by their biochemical properties using standard method. Azotobacter shows all positive IMVIC test whereas Rhizobium show all negative IMVIC test. The viability of isolate is calculated that is 81.45% in azotobacter and 52.2% in rhizobium hence this indicates azotobacter is more tolerant to pesticide as compare to rhizobium.At neutral pH both the isolate having greater density and growth.

3. References:

1) Sonam Shaheen1 and S. Krishan Sundari1(2013) ''Exploring the applicability of PGPR to remediate residual organophosphate and carbamate pesticide used in agricultural field"
2) International Journal of Agriculture and Food Science Technology.
3) ISSN 2249-3050, Volume 4, Number 10 (2013), pp. 947-954
4) MuneesAhemada, b,*, MulugetaKibretb (2014)''Mechanisms and applications of plant growth promotingrhizobacteria: Current perspective''. Journal of king Saud University.
5) Najam-ul-Sehar, Maqshoof Ahmad*, Muhammad Fakhar-u-ZamanAkhtar, MoazzamJamil,
6) Muhammad Latif and Iqra Ahmad (2015) ''Pesticide tolerant plant growth promoting rhizobacteria isolated from rhizosphere of okra' University College of Agriculture and Environmental Sciences, the Islamic University of Bahawalpur
7) Durgeshkumarjaiswal, Vikrant kumar, Jay prakashverma* AND Janardanyadav2(2013)
8) Isolation and characterization of strain from pesticide contaminated site of uttarpradeshInstitute of Environment and Sustainable Development, Banaras Hindu University, Varanasi-221005, Uttar Pradesh, India
9) André LuizMeleiro Porto, GliseidaZelayaránMelgar,Mariana ConsiglioKasemodel and Marcia Nitschke''Biodegradation of Pesticides''UniversidadedeSãoPaulo, InstitutodeQuímicadeSãoCarlos,Brazil
10) Poorva G. Ghosh; Neha A. Sawant; S. N. Patil; B. A. Aglave (2010); Microbial biodegradation of organophosphate pesticide' International Journal of Biotechnology and Biochemistry; Vol 6 (6) pp. 871-876
11) M Ahemad and M S Khan (2011), Assessment of pesticide-tolerance and functional diversity of bacterial strains isolated from rhizospheres of different crops, *Insight Microbiol,* **1,** pp.8-19.
12) N a s t a s i j a B. M r k o v a å k i, N i k o l a A. Å a å i ã ,V e r a M . M i l i ã (2002) '' Effect of pesticide on azotobacterchroccocum''Institute of Field and Vegetable Crops, Novi Sad, MaksimaGorkog 30, Yugoslavia

13) Ke Liu (2015)Selecting Plant Growth-Promoting Rhizobacteria (PGPR) for Both Biological Control of Multiple Plant Diseases and Plant Growth Promotion in the Presence of Pathogens Auburn, Alabama