To Study The Effect of Pesticide Resistant Azotobacter Spp. on Plant Growth

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Abstract- *Azotobacter spp. is Gram negative free living nitrogen fixing bacteria and also able to provide essential chemicals to plants like IAA, Siderophore, HCN, Ammonia, Nitrogen through their activities. Now days Large No. of Bio-fertilizers are available, they give better result at lab level but they failed at actual field level because some factor like, already used chemical pesticide and other environmental condition. Chemical pesticides residues remains in the soil, due to these micro-organisms are unable to grow because of these bio-fertilizers not give better result at actual field level. So to overcome to this problem current study was done on isolation on pesticide resistant Azotobacter from the agricultural field which is pre-exposed by chemical pesticide and their effect was checked on seed germination. Ashby's medium containing different concentration of pesticide used for isolation of pesticide resistant Azotobacter spp. Relevant bio-chemical and plant growth promoting traits of isolate was studied. Seed germination effect of pesticide resistant Azotobacter was checked and combine effect of bio-fertilizer and chemical fertilizer on seed germination also checked.*

Keywords- Bio-fertilizers, Azotobacter spp., plant growth promoting traits, combine effect of bio-fertilizers and chemical fertilizers.

I. 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. Bio-fertilizers are the living cells or latent cells of efficient strains of micro-organisms that help crop plants to uptake nutrients by their interaction in the rhizosphere when applied through seed or soil [4]. Microbial inoculants generally called as "Bio-fertilizers", are carrier based preparations containing beneficial microorganisms in a viable state intended for seed or soil application designed to improve soil fertility and to help plant growth by increasing the number and biological activity of desired microorganisms in the root environment (SubbaRao, 1993). *Azotobacter spp.* have various plant growth promoting traits like Nitrogen fixation, IAA production.Now a days there is a large production of bio-fertilizers. Though they give very good result as lab level, but they failed at actual field level. There are many factors behind it, like competition of bio-fertilizer with native soil flora, non-adaptability of bio-fertilizer in the soil environment, unsuitable environmental condition for the growth of bio-fertilizers. The accumulated salt and pesticide residue is one of important factors for the ineffectiveness of bio-fertilizers.The high use of chemical pesticides and chemical fertilizers may affect the soil micro-organisms. The pesticide residues and fertilizers residues which present in soil affect the bio-fertilizer activity [9]. There is about 0.25 to 0.35% pesticide concentrations present in Indian soil [8]. Such high concentration of chemical pesticide harms soil micro-organisms, chemical fertilizer residues also harm to soil micro-organisms along with applied bio-fertilizer and decreases its plant growth promoting activities like IAA production, Nitrogen fixation and other [9].To overcome this problem present study was focused on isolation of pesticide and salt resistant *Azotobacter* from agricultural soil which are pre-exposed to chemical pesticide and chemical fertilizer.

For this purpose following objectives were put forth:

1)Isolation of pesticide resistant bacteria from soil sample. 2) Characterization andidentification of the isolates. 3) Study of plant growth promoting traits of the isolates. 4) Comparison studies to check the effect of biofertilizer on seed germination.

II. 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.

Isolation Of Pesticide Resistant Bacteria:- 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 . Inoculated flasks were incubated at 37^{o}C for 72-96 hrs. (3-4 days). After the incubation, flasks were observed for turbidity. After 72 hrs ,a loopful of culture was streaked onto sterile Ashby's agar media 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. Inoculated plates were incubated at 37^{o}C for 72-96 hrs. (3-4 days). After the incubation, the plates were observed for growth.

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, H_2S production, Urease test, Sugar fermentation like Glucose, Sucrose, Lactose, Maltose etc.

Catalase Test:- 3% solution of hydrogen peroxide was taken in the test tubes and individual isolated colony was immersed into the tube and observed for effervescence.

Oxidase Test:-A loopful culture of organism was streaked on filter paper strip previously dipped in 1% aqueous solution of N, N, N, N tetra methyl Paraphenylenediaminedihydrochloride (oxidase reagent) and observed for formation of blue color within 10 seconds.

Capsule Demonstration:-Capsule demonstration was performed by using Maneval's method.

Sugar Fermentation:-Isolate was assessed for its sugar fermentation ability. The isolate was inoculated separately in different tubes containing the sterile peptone water supplemented with 1% of glucose, sucrose, lactose and maltose respectively with phenol red as pH indicator and an inverted Durham's tube. Uninoculated sterile peptone water was kept in a test tube as control. After incubation at 37^{0}C for 24hrs, the tubes were observed for acid and gas production.

Hydrogen Sulfide (H_2S) Production Test:-Isolate was assessed for its H_2S production. The isolate was inoculated in sterile TSI agar slant with phenol red used as pH indicator. The uninoculated sterile TSI agar slant was kept as control. After incubation at 37^{0}C for 24 hrs, the tube was observed for H_2S production.

Urease Test:-Isolate was assessed for its urease production. The isolate was inoculated in sterile urease agar slant. The uninoculated sterile urease agar slant was kept in test tube as control. After incubation at 37^{0}C for 24 hrs, the tube was observed for urease production

Assessment Of Plant Growth Promoting Traits Of Isolate:

Indole Acetic Acid Production:-0.1 ml of 24 hrs old culture of isolate was aseptically inoculated in a sterile nutrient broth containing 0.1% of tryptophan. Another sterile uninoculated nutrient broth was kept in another test tube as a control. Both the test tubes were incubated for 24 hrs at 37^{0} C. After incubation, 2ml of sample from test and control were centrifuged at 10,000 rpm for 10 minutes. 0.5 ml supernatant from each tube was mixed with 2ml of Salkowaski reagent and tubes were incubated at room temperature in dark for 30 minutes and observed for development of cherry red color [7].

Siderophore Production:-Siderophore production of isolate was studied by CAS liquid assay method. 0.1 ml of 24hrs old culture of isolate was aseptically inoculated in 5ml of sterile succinate broth, and 5 ml of sterile uninoculated succinate broth was kept in another test tube as control. Both test tubes were incubated for 24 hrs at 37^{0}C. After incubation, 2 ml sample from test and control were centrifuged at 10,000 rpm for 10 minutes. 0.5ml supernatant from each tube was mixed with 2ml of CAS reagent and observed for reduction in blue color [1].

HCN Production:-24 hrs old culture of isolate were aseptically streaked on sterile nutrient agar plate containing 0.45% glycine. A circular disk of Whatmann filter paper was soaked in 0.5% of picric acid solution + 0.2 sodium carbonate solution and disk was kept in lid of petriplate. Plate was then incubated at 37^{0}C for 5 days. After incubation, plate was observed for development of brown color on filter paper [5].

Ammonia Production:-0.1 ml of 24 hrs old culture of isolate was aseptically inoculated in 5ml sterile peptone broth. Another test tube containing 5ml uninoculated sterile peptone broth was kept as control. Both tubes were incubated at 37^{0}C for 5 days. After incubation, 0.5 ml Nessler's reagent was added to each tube and observed for development of brown color [2].

Antifungal Activity:-Antifungal activity of the culture was determined by using agar well diffusion method. The isolate was inoculated in sterile nutrient broth and incubated at 37^{0}C for 48 hrs. Then 2ml culture was centrifuged at 8,000 rpm for 10 minutes. 24 hrs old culture of each test fungi (*Aspergillus niger, Aspergillus flavus, Alternaria alternata*) was spread aseptically on sterile Sabouraud agar plates and immediately

the wells were prepared on the plate. Then 100µl of cell free supernatant of isolate was added in each well. The plates were then kept for diffusion in refrigerator and then incubated 37^0C for 24-48 hrs. After incubation, plates were observed and diameters of zones of inhibition were measured [3].

Effect Of Bio-Fertilizer On Seed Germination:-Effect of Bio-fertilizer on seed germination was performed by following methods:

Effect Of Bio-Fertilizer On Seed Germination In Petriplate:-For the test, sterile petriplates with sterile filter paper was used. 15 healthy wheat seeds were selected. After selection of seeds, they were socked in 1% sodium hypochlorite for 10 minutes. After the treatment of 1% sodium hypochlorite, the seeds were washed with sterile D/W for 2-3 times to remove 1% sodium hypochlorite. These washed seeds were equally distributed in 3 plates (control, bio-fertilizer and chemical fertilizer respectively), each plate contain 5 seeds of wheat. For Bio-fertilizer and chemical fertilizer, the seeds were dipped in 10ml of Bio-fertilizer (5 seeds) and chemical fertilizer (5 seeds) for 10 minutes. For control, 5 seeds were dipped in sterile D/W and placed in sterile petriplate which contains sterile filter paper. These treated seeds were placed in different sterile perti plates containing sterile filter paper. Sterile D/W was added in petriplate on filter paper to maintain moisture level. Then plates were incubated in incubator at 37^0C.Afetr each day sufficient moisture was maintained for upto 7 days. After 7 days the root and shoot length of seeds were measured.

Combined Effect Of Bio-Fertilizer And Chemical Fertilizer:-For the test sterile petriplates containing sterile filter paper was used. 20 healthy wheat seeds were selected. After selection of seeds, they were socked in 1% sodium hypochlorite for 10 minutes. After the treatment of 1% sodium hypochlorite, the seeds were washed with sterile D/W for 2-3 times to remove 1% sodium hypochlorite. For the test, various combinations of bio-fertilizers and chemical fertilizers were used (80+20, 70+30, 60+40% respectively) these washed seeds were equally distributed in 4 plates (control, bio-fertilizers plus chemical fertilizer ratio respectively), each plate contained 5 seeds of wheat. For control, 5 seeds were dipped in sterile D/W and placed in sterile petriplate which contains sterile filter paper. For combination of 80 + 20% ratio, the 5 seed of wheat were treated in 80 + 20% ratio of bio fertilizer and chemical fertilizer for 10 minutes. This step was repeated for 70 + 30% and 60 + 40% ratio respectively. These treated seeds were placed in different sterile petri plates containing sterile filter paper. Sterile D/W was added in petriplate on filter paper to maintain moisture level. Then plates were incubated in incubator at 37^0C. After each day sufficient moisture was maintained for upto 7 days. After 7 days the root and shoot length of seedswere measured.

Effect Of Bio-Fertilizer On Seed Germination In Pot Experiment:-For the test plastic pots were used which were filled with sterile soil in it. 60 healthy wheat seeds were selected. After selection of seeds, they were soaked in 1% sodium hypochlorite for 10 minutes. Then the seeds were washed with sterile D/W for 2-3 times to remove 1% of sodium hypochlorite. For control, 20 seeds were placed in plastic pots (4 seeds/ pot) after dipping them in D/W. For bio fertilizer and chemical fertilizers, the seeds were dipped in 10 ml of bio fertilizer (20 seeds) and chemical fertilizers (20 seeds) for 10 minutes. These treated seeds were sowed in plastic pots(4 seeds/pot). The water was added in the pots to maintain the moisture level required for the seed germination and then kept in sunlight condition. The moisture level was maintained up to 15 days by adding water.

Combined Effect Of Chemical Fertilizer And Bio-Fertilizer:-For the test plastic pots were used which were filled with sterile soil in it. 80 healthy wheat seeds were selected. After selection of seeds, they were soaked in 1% sodium hypochlorite for 10 minutes .Then the seeds were washed with sterile D/W for 2-3 times to remove 1% of sodium hypochlorite. For the test, various combinations of Bio-fertilizer and chemical fertilizers were used (80+20, 70+30, 60+40% respectively). For control, 20 seeds were placed in plastic pots (4 seeds/ pot) after dipping them in D/W. For combination of 80+20% ratio of Bio-fertilizer and chemical fertilizer, the 20 seeds of wheat were dipped in a solution of 80+20% ratio of bio-fertilizer and chemical fertilizers for 10 minutes. This step was repeated for 70+30% and 60+40% ratio respectively. These treated seeds were sowed in soil in different plastic pots (4 seeds/pot). The water was added in the pots to maintain the moisture level required for the seed germination and then kept in sunlight condition. The moisture level was maintained up to 15 days by adding water.

III. RESULTS

Isolation of Pesticide Resistant Bacteria From Soil Sample:- After 72 hrs 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 72 hrs 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. **.3 Morphological Characterization:-**The Colony characteristics were recorded as follows:

Table No. 1:-Colony characteristics

Size	2mm
Shape	Circular
Color	Pale yellow
Elevation	Convex
Margin	Entire
Opacity	Opaque
Consistency	Sticky
Gram Character	Gram Negative rod

Fig.No.1:-Isolate

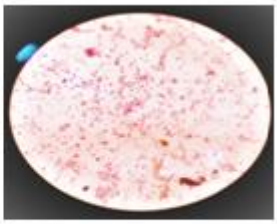

Fig.No.2:-Gram

Biochemical Characterization Of Isolates:- Biochemical characterization of isolate was performed. The isolate was catalase and oxidase positive.

Capsule demonstration: - The colorless capsule was observed.

Sugar fermentation test:-After 24 hrs incubation, for all the sugars tested, the color change was observed from pink to yellow and gas production also observed, that indicate the isolate was able to ferment glucose, lactose, maltose and sucrose with acid and gas production.

Hydrogen sulphide test:-After 24 hrs incubation the color change was observed from pink to black color that indicate the production of Hydrogen sulphide and the position of slant also changed that indicate the production of gas.

Urease test:-After 24 hrs of incubation the color change was observed from yellow to orange which indicates the hydrolysis of urea.

The isolate was a Gram negative motile short rod, catalase and oxidase positive and also able to ferment glucose, sucrose, lactose and maltose, it produced urease as well as H_2S. It was grown on Ashby's media .Hence according to Bergey's Manual of Determinative Bacteriology; the isolate was tentatively identified as an *Azotobacter* species [10].

Assessment of plant growth promoting traits:-

Indole Acetic Acid production:-When 0.5 ml of cell free broth of isolate was mixed with 2ml of salkowaski reagent and incubated for 30 minutes in dark, cherry red color was developed. This indicated that the isolate was able to produce indole acetic acid.

Siderophore production:-After incubation in succinate, when 0.5 ml of cell free broth of the isolate was mixed with 2ml of CAS reagent, a reduction in blue color was observed which indicated that isolate have ability to produce siderophore

HCN production:-After incubation on nutrient agar plate containing 0.44% glycine, brown color developed on filter paper. It indicated that isolate was able to produce HCN.

Ammonia production:-When isolate were inoculated in peptone water and incubated for 5 days a dark brown color was developed after addition of few drops of Nessler's reagent. This indicated that isolate was able to produce ammonia.

Antifungal activity:-After incubation, zones of inhibition were observed against any test fungi using isolate which indicated that isolate was able to inhibit the growth of test fungi.

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

Effect of treatment with Biofertilizer on seed germination in petriplate:-

For the effect of treatment with Biofertilizer on seed germination in petriplate, the bio-fertilizer was prepared by inoculating pure culture of *Azotobacter spp.* in sterile Ashby's broth which was incubated at 37^{o}C for 48- 72 hrs. This culture was used as bio-fertilizer. After 7 days root and shoot lengths were measured. The observed root and shoot length was

statistically analyzed by ANOVA single factor test using Microsoft excel software as follows:-

Table No. 3: Wheat root length

Seed No.	Wheat Root length (cm)		
	Control	Chemical Fertilizer	Bio-Fertilizer
1	1.0	1.5	1.95
2	0.95	1.15	2.05
3	No growth	0.25	1.85
4	No growth	No growth	2.05
5	No growth	No growth	No growth

ANOVA Single Factor test:-

The P value after performing ANOVA test was **0.048726.**

Level of significance (α value):- **0.05**

As **P value** isbelow the Level of significance (α value) it indicated that there is significant difference between the treatment of Bio-fertilizer and Chemical fertilizer on the height of wheat root.

So it was concluded that Bio-fertilizer shows better result than Chemical fertilizer

Table No. 4:- Wheat shoot length

Seed No.	Wheat shoot length (cm)		
	Control	Chemical Fertilizer	Bio-Fertilizer
1	2.1	2.4	5.2
2	2.2	3.1	4.9
3	No growth	1.1	6.7
4	No growth	No growth	5.7
5	No growth	No growth	No growth

ANOVA Single Factor test:-

The P value after performing ANOVA test was **0.025699.**

Level of significance (α value):- **0.05**

As **P value** below the Level of significance (α value) it indicated that there is significant difference between the treatment of Bio-fertilizer and Chemical fertilizer on the height of wheat shoot.

So it was concluded that Bio-fertilizer shows better result than Chemical fertilizer.

Combine effect of Bio-fertilizer and chemical fertilizer:-

The combine effect of Bio-fertilizer and chemical fertilizer on germination on wheat seeds was studied. But no growth was observed, growth was observed in soil pot experiment

Effect of biofertilizer on plant growth in pot experiment:-

Effect of bio-fertilizer on germination of wheat seeds in soil was studied. After 10 and 15 days the height of wheat crop were measured. The observed height of wheat crop as follows,

Table No. 5:-Effect of biofertilizer on plant growth in pot experiment

Pot no.	Height of plant after 15 days in cm		
	Control	Chemical fertilizer	Bio-Fertilizer
1	10.0	12.0	14.2
2	10.2	12.4	15.0
3	11.0	11.0	15.2
4	11.4	12.5	14.0
5	11.2	13.0	12.2

ANOVA Single Factor test:-

The P value after performing ANOVA test was **1.89E-08.**

Level of significance (α value):- **0.05**

As **P value** below the Level of significance (α value) it indicated that there is significant difference between the treatment of Bio-fertilizer and Chemical fertilizer on the height of wheat crop.

So it was concluded that Bio-fertilizer shows better result than Chemical fertilizer.

Combine effect of biofertilizer and chemical fertilizer on plant growth in pot experiment:-

The combine effect of bio-fertilizer and chemical fertilizer on germination on wheat seeds was studied. After 10 days and 15 days the height of wheat crop was measured. The observed height of wheat crop as follows:

Table No. 6:-Combine effect of biofertilizer and chemical fertilizer on plant growth in pot experiment:-

Pot no.	Height of plant after 15 days (cm)			
	Control	80+20	70+30	60+40
1	10.0	11.0	12.0	14.0
2	11.0	13.0	12.9	14.1
3	10.2	14.5	13.0	13.9
4	11.4	13.1	12.5	14.4
5	11.2	12.9	11.8	14.8

ANOVA Single Factor test:-

The P value after performing ANOVA test was **2.64E-05.**

Level of significance (α value):- **0.05**

As **P value** below the Level of significance (α value), it indicated that there is significant difference between the combine treatment of Bio-fertilizer and Chemical fertilizer on the height of wheat crop.

So it was concluded that the **60+40%** ratio of Bio-fertilizer and chemical fertilizer shows better result than any other combination.

Thus, from the above statistical analysis of the effect of biofertilizer on seed germination, it was concluded that the Azotobacter spp. as a biofertilizer enhanced seed germination.

IV. 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.The chemical pesticide residues present in soil affect many soil microbes; pesticide residues affect the plant growth promoting activity of bio-fertilizers [9]. In the present study, a pesticide resistant bacteria was isolated from agricultural field soil which can resist upto 3% pesticide. Shaheen,S. and Krishnna, S. (2013) isolated the pesticide resistant PGPR which can resist the pesticide

Figure No.3:- Antifungal activity

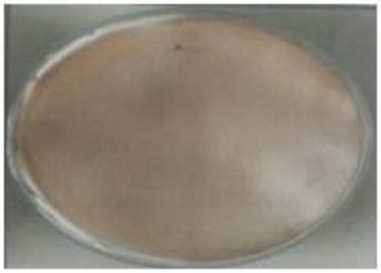

Figure No.4:-HCN production

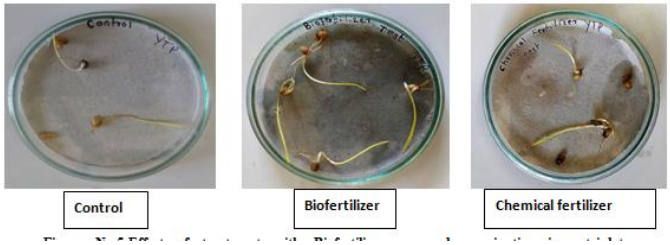

Figure No.5:Effect of treatment with Biofertilizer on seed germination in petriplate

concentration from 10ppm to 100 ppm of pesticide Monocrotophos and Dimethoate [8].. The NPK salt residues present in soil also affect many soil microbes, salt residues affect the plant growth promoting activity of bio-fertilizer Md. ShamimAkhter, ShaikhJulfikarHossain, SK. Amir Hossain, Rakesh Kumar Datta (2012) isolated the salinity tolerant *Azotobacter spp.* which can resist the upto 1.5% salt concentration [6]. In the present work a salt resistant *Azotobacter* was isolated from agricultural field soil which can resist upto 1.5% salt concentration. The pesticide and salt resistant isolate have different plant growth promoting activities like nitrogen fixation, IAA production, siderophore production etc. Antifungal activity was also shown by the isolate.The effect of bio-fertilizer on wheat crop was studied. In this present study,it was found that the height of the wheat crops in test was better than in control and after treatment with chemical fertilizer. Bio-fertilizer inoculated wheat crops showed the more greenish color than the control wheat crops. The similar results were obtained in another study by K.S. Gomare, M. Mese, and Y. Shetkar (2013) .They studied effect of bio-fertilizer on legume and non-legume plants. They found that the productivity can potentially be improved through the use of bio-fertilizer, it resulted in the most of inoculated pots

plants color found to be greenish with proper growth as compared to control.

The comparative effect of bio-fertilizer and chemical fertilizer on wheat crop was studied.But in combined effect of biofertilizer and chemical fertilizer shows the better results. Sanjay Mahato, AsmitaKafle (2018) carried out comparative study of *Azotobacter* with or without other fertilizers on growth and yield of wheat. They found that only *Azotobacter* bio-fertilizer is not as effective as in combination with in organic fertilizers. Seed inoculated *Azotobacter* shows a better result than soil inoculated *Azotobacter*. When *Azotobacter* is used without inorganic fertilizer, the yield increases is much higher (43%). Interestingly, when *Azotobacter* is used with a mixture of inorganic fertilizer, the yield increases is maximum (63%). The combined effect of bio-fertilizer and chemical fertilizer on growth of wheat crop was studied.In present study it was found that the plant shows maximum height (14.80 cm after 15 days) for combination of 60+40 % ratio of bio-fertilizer + chemical fertilizer , it was also followed by 70+30% and 80+20% ratio of bio-fertilizer and chemical fertilizer and shows the height (13.00 cm and 14.50 cm after 15 days) respectively. It was found that the better result in 60+40% ratio of bio-fertilizer and chemical fertilizer. Sanjay Mahato, AsmitaKafle (2018) carried out comparative study of *Azotobacter* with or without other fertilizers on growth and yield of wheat. They found that the plant height shows the significant result with the different treatments. The plant shows maximum height (79.88 cm after 74 days) with the combination of *Azotobacter* inoculated seed + NPK + FYM followed by T5 with inoculated seed + NPK (76.9 cm) and T2 with soil + NPK (76.5 cm) that is combination of inoculated seed + NPK + FYM shows the better result and height.

V. 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.

The pesticide resistant *Azotobacter spp.* isolated in this study has multiple plant growth promoting traits which included nitrogen fixation, IAA production, siderophore production, HCN production, Ammonium production and Anti-fungal activity; however it lacked ability to solubilize phosphate.

The effect of pesticide and salt resistant biofertilizer was studied on the growth of wheat crop. The isolate was able to increase the height of wheat crop. The biofertilizer treated wheat seeds showed the high height as compared with the control wheat seeds. The pesticide resistant biofertilizer was able to increase the height of crops as well as inoculated pot plants color was found to be more greenish as compared to the control. Thus for the increased yield of crops, the isolated *Azotobacter spp* as a biofertilizer may be a good option.

The combine effect of Bio-fertilizer and chemical fertilizer on the wheat crop was tested when Bio-fertilizer was used with a mixture of chemical fertilizer, the plant height increased significantly as compared to when only Bio-fertilizer was used. Hence, combined use of Bio-fertilizer and chemical fertilizer is good option to increase the productivity of plants.

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 pesticides.

VI. ACKNOWLEDGEMENT

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