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## ASSESSMENT OF STUDENTS KNOWLEDGE AND PERCEPTIONS ABOUT BIODIVERSITY AND CONSERVATION METHOD IN HARARI REGIONAL STATE, EASTERN ETHIOPIA

Yeneayehu Fenetahun<sup>1\*</sup> and Girma Eshetu<sup>2</sup>

*Ethiopia Biodiversity Institute (EBI), Harar Biodiversity Center*

*Email: [yeneayehu07@gmail.com](mailto:yeneayehu07@gmail.com)*

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**Abstract:** The term biodiversity refers the number and variability of living organisms on the plant and it is the heart of sustainable development and the life insurance in itself. The main objective of this study was to assess students' level of knowledge and perception about biodiversity conservation techniques, hence strengthening and developing students' level of knowledge and perception towards biodiversity conservation has a great role to protect the variety of all species in the ecosystem. The study has employed both qualitative and quantitative methods such as individual interview, FGD and structured questionnaire. A total 360 students from two target groups (grade 10 and 12) selected from 6 secondary and preparatory schools were involved. The results showed that students' level of knowledge and perception towards biodiversity conservation was varied. Accordingly, above 50% and 70% of the students of grade 12 were found above mastery level in their knowledge and had shown favorable perception respectively regarding biodiversity conservation whereas students from grade 10 above 50% were found below mastery level regarding their knowledge and above 50% of the students also had shown favorable perception about conservation of biodiversity resource. This indicated that the students were not more awareness about biodiversity and conservation methods due to different factors like teaching learning of biodiversity conservation was found ineffective due to lack of facilities, lack of effective implementation of the stated methodology in their text book and large class size. Thus, it can be concluded that the students has not get the expected change in knowledge and perception among students about conservation of biodiversity resources particularly in grade 10 with in the school. Therefore, fulfilling of the necessary facilities, awareness creation on the concerning body and implementing effectively the teaching methods of biodiversity conservation that included in their text book such as field exposure, group discussion active classroom session and continues assessment in the study area is highly recommended.

**Keywords:** Biodiversity, Conservation, Harari, Students, Perception

### INTRODUCTION

The term biodiversity refers the number and variability of living organisms. It includes diversity within species (genetic diversity), between species (species diversity), and between ecosystems (ecosystem diversity). Biodiversity also incorporates human and cultural diversity, which can be affected by the same drivers as biodiversity, and which has impacts on the diversity of genes, species and ecosystems (Niles, 2009). Biodiversity is the heart of sustainable development and the life insurance in itself (Mc Neil and Shei, 2002, cited in Sajise, 2005). According to FAO (1995) cited in Young (1997), sustainable development is the management and conservation of the natural base in such a manner as to ensure the attainment and continued satisfaction of human needs for the present and the future generations.

Since it is crucial to the sustainability of sectors as diverse as energy, agriculture, forestry, fisheries, wildlife, industry, health, tourism, commerce, irrigation and power the conservation of biodiversity is fundamental to achieving sustainable development. Ethiopia's development in the future will continue to depend on the foundation provided by living resources and conserving biodiversity (NBSAP,

2005). Biodiversity conservation in Ethiopia will be better served, at least initially, by a distinctive and focused action plan. Such a plan can promote awareness, knowledge and perception of the society. According to Getachew and Berihun (1996 E.C), especially countries like Ethiopia that have people whose life is directly connected with the biodiversity (natural resources) would know the benefit of the biodiversity. Since Ethiopia is characterized by wider ranges of landscapes starting from below the sea level at Afar Depression up to 4200 m a s l at Ras Dashen and different climatic zones, the combination of which has contributed much to the diversity of both plant and animal communities (Getachew and Berihun, 1996, Lavrenchenko *et al.*, 2004). In addition, Ethiopia is also a home of many nations and nationality which possess experiences, diverse culture and knowledge that can play a great role in conserving the biodiversity of the nation (Getachew and Berihun, 1996). Ethiopia is an important center of biodiversity and endemism on the African continent. An inventory of fauna and flora in Ethiopia indicates that there are more than 277 species of terrestrial mammals, 862 species of birds, 201 species of reptiles, 63 species of amphibians, 150 species of fish and 7000 species of higher plants. Among these, 11% of mammals, 3.3% of birds, 4.5%

\*Corresponding Author

of reptiles, 38% of amphibians, and 12% of higher plants are endemic (EFAP, 1994). The contracting parties of 1992 UNCBD in Riode Janeiro recognized that the general lack of information and knowledge about biodiversity has been one of the leading causes for reduction and loss of biodiversity.

Since Ethiopia is one of the contacting parties of the convention that endorsed and ratified the convention protocol (UNCBD, 1992), the government of Ethiopia has been carrying out many activities to control and overturn the situation that is to conserve and develop biodiversity. One of the control measures that have been under taken by the government is to educate citizens through formal school program about concepts, benefits and problems of biodiversity in the hope that individuals will recognize the long-term values of biodiversity for survival and get involved in conservation activities. To promote this, biodiversity contents, objectives, instructional methods and materials are integrated with biology syllabi of grade 7 to 11 (Wendye, 2009). Recently it also included in grade 12 biology textbook (MoE, 2009). Several authors such as Humston and Ortiz-Barney (2007); Leeming *et al.*, (1993); Rickinson (2001) and Zelezny (1999) have shown that content coverage of environmental issues and ecological principles increases student awareness, and positively affects attitudes, behaviors, and values regarding conservation issues. According to Ammannuel (2014) peoples having low level of knowledge and perception of biodiversity conservation are the major threats of biodiversity. The IUCN's (2007) Red List shows that Ethiopia has 6 species that are critically endangered, 22 endangered and 70 vulnerable. And lack of awareness and knowledge on the part of the people about biodiversity is one of the major factors that contribute for loss of biological resources (MoE, 1988). The loss of biodiversity like logging, hunting, fire wood collection, forest fire because of fire for a honey collection or caused due to smoking cigarettes carelessly and timber felling occurred due to low knowledge and perceptions of peoples towards biodiversity conservation (Manuel *et al.*, 2006).

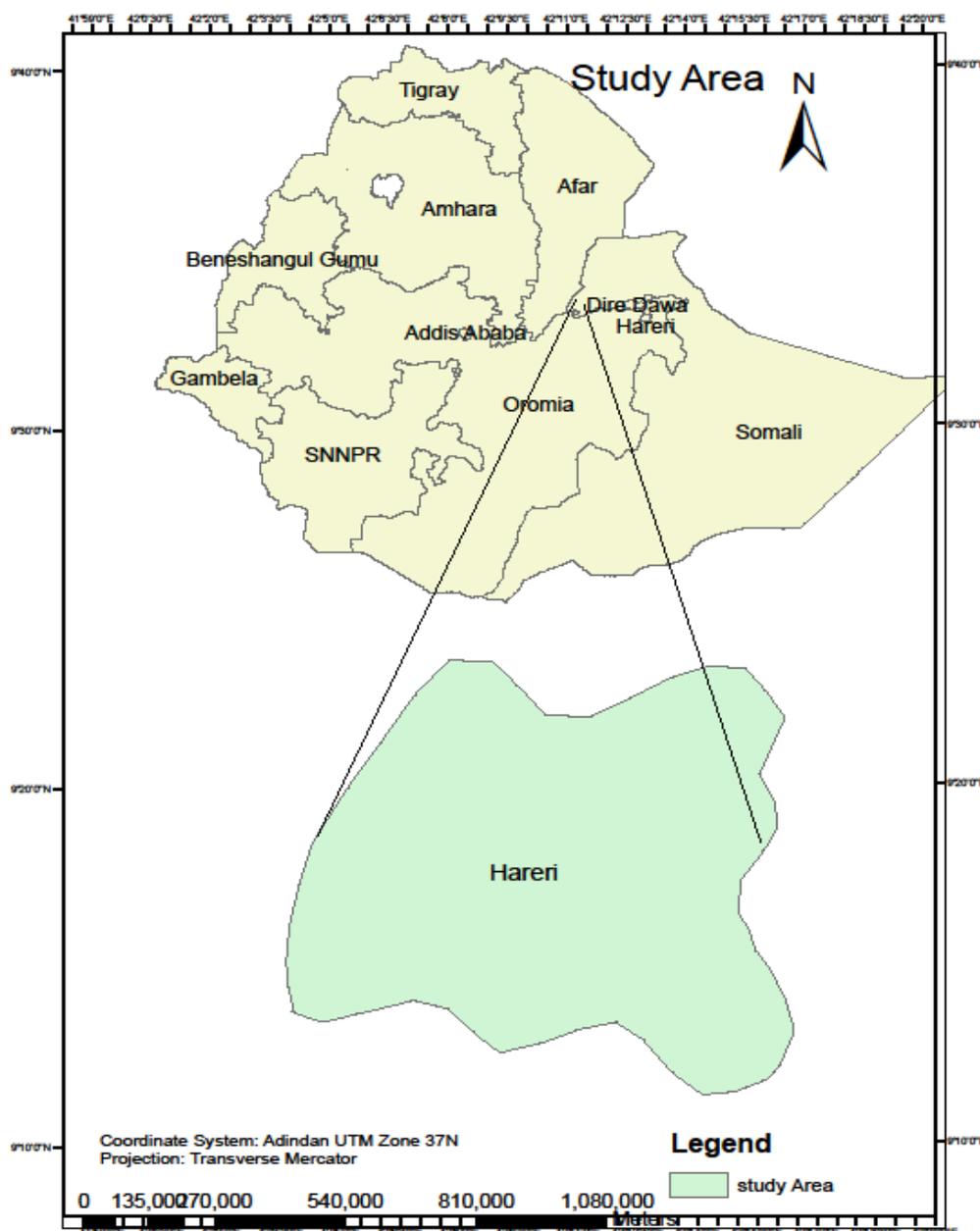
According NBSAP (2005) the biodiversity conservation is very important since the human being is widely benefited from the biodiversity and it is fundamental for the development of socio-economic

and stable environment. Students that have good knowledge and perceptions about the benefit of the biodiversity will develop good attentions for the conservation of biodiversity. According to Wendeye (2009) study the case of some preparatory school in South Wollo Zone particularly in grade 11, the majority of the students' knowledge to biodiversity is scored below the required level or mastery level (50%) for students in preparatory school particularly grade 11 and this indicate that, it needs to work hard to develop students' knowledge towards biodiversity which help to conserve the biological resources. In harari regional State eastern Ethiopia so far no obvious research has been done regarding students' level of knowledge and perceptions about biodiversity and conservation methods. Therefore, the purpose of this study was to assess students' level of knowledge and perceptions and to suggest further recommendation about biodiversity, its threatening factors as well as appropriate conservation methods in Harari Regional state eastern Ethiopia.

## MATERIAL AND METHOD

### Study Area

The study was conducted in Hareri regional state, East Ethiopia (Fig 1.). Harari is one of nine national regional states in Ethiopia having nineteen kebeles (lower administrative level). The study area was located 525km far from Addis Ababa which is the capital city of Ethiopia. Geographically, the area is located between  $42^{\circ}03' 30''$ -  $42^{\circ}16' 24''$ E and  $9^{\circ}11' 24''$ N with an altitude ranging from 1300-1600 m.a.s.l. The mean annual rainfall the area is 636.7mm and the mean annual temperature is  $19^{\circ}$ C. The total population of the region is estimated to 238,000 which of 120,000 males 118,000 females. Generally the region has a total area of 334km<sup>2</sup>. It also home to diverse ethnic groups. The Oromo, Amhara and Adare are the major ethnic group living in the study area. Each ethnic group has their own composition of with distinctive language (e.g., Oromifa, Amharic and Harari), and cultural diversity. Islam is the predominant religion with 69% of the population reporting themselves as adherents of the religion, followed by Orthodox Christianity (27%) (Zelezny; 1999).



**Fig 1.** Map of study area, Hareri, Ethiopia

**Design of the Study**

The design of the study was a mixed methods design (cross-sectional descriptive survey). The descriptive survey was used to collect information from the respondents and assess student’s knowledge and perception about biodiversity conservation.

**Study Population Sampling Technique**

This assessment was addressed to students from six (6) secondary and preparatory school and from this Four (4) of them were government school (2<sup>nd</sup> model, Abubeker, Junnier and Yeshimebet secondary and preparatory school) and Two (2) of them were private school ( Betelehem and Zoomin secondary

and preparatory school) in Harari Regional State eastern Ethiopia. Two target groups were considered in this study :( 1) grade 10 students and (2) grade 12 students. The assessment data were collected from students through semi-structure questionnaire, face to face interview and focus group discussion based methods given by (Halford *et al* ;2011). First Stratified random sampling was used based on their grade level to group in to two strata then Random sampling technique was used to select those study participants among grade 10 and 12 natural science students. The sample size was depends on

availability of time and budget rather than the total population.

A total of 360 assessment data were collected from 360 interviewers (240 male and 120 female) from each school 60 students (40 male and 20 female) and 30 from grade 10 ( 20 male and 10 female) and 30 from grade 12 ( 20 male and 10 male). The assessment was conducted from Jan, 2016 to May, 2017 at respondent’s school.

**Data analysis**

To answer the research questions of this study, quantitative and qualitative data were collected and analyzed using both quantitative and qualitative data techniques. The quantitative data were, tabulated and analyzed using frequency and percentage. The description of the data collected by interview, class room observation and focus group discussions were incorporated into the quantitative data where they support each other and the content analysis was analyzed qualitatively using the identified key concepts.



*Photo during explanation about the question and its target to students and during students answer the question (photo taken by Yeneayehu Fenetahun, Feb/2017)*

**RESULT AND DISCUSSION**

All the data that assessed students’ level of knowledge and perception towards biodiversity and

conservation techniques were summarized and presented in table form.

**Assessment of Students ‘Level of Knowledge about Biodiversity Conservation (both open and closed ended question)**

To assess the level of knowledge about biodiversity respondents were asked to respond both the close ended questionnaire by selecting Yes/No/Not sure and open ended knowledge questions such as defining biodiversity and biodiversity conservation, Identifying threats to biodiversity, describing the importance of biodiversity conservation etc. To identify the level of knowledge of the students about biodiversity conservation below mastery level (i.e. below 50%) and above mastery level (above 50%)

was used (Wendeye, 2009). This means if almost all the items are responded by majority of the students, the majority students' level of knowledge (i.e. above 50% of the students) are above the mastery levels (i.e. above 50%) and if almost all the question items are responded incorrectly by majority of the students, the majority students' level of knowledge (i.e. above 50% of the students) are below the mastery level (i.e. below 50%).

**Closed ended question result data**

**Table 1.** Grade 10 students' (N=180) level of knowledge about biodiversity conservation (close ended questionnaire).

Question type	Respondent responses		
	Yes	No	Not sure
	F (%)	F (%)	F (%)
1. Biodiversity is the extent to which an ecosystem contains different species	101 (56.1)	72 (40)	7 (3.9)
2. Biodiversity uses as a human well-being	131 (72.8)	45 (25)	4 (2.2)
3. Biodiversity conservation refers to the practice of protecting and preserving the abundance and variety of all species.	79 (43.9)	96 (53.3)	5 (2.8)
1. Deforestation has negative impact on the biodiversity resources.	151 (83.9)	28 (15.5)	1 (0.6)
2. It is always better to repair an ecosystem rather than to replace it.	83 (46.1)	76 (42.2)	21 (11.7)
3. Climate change cannot control through biodiversity conservation	111 (61.7)	60 (33.3)	9 (5)
4. Protection of species and varieties of species will not support biodiversity.	119 (66.1)	49 (27.2)	12 (6.7)
5. Conservation of biodiversity resource has negative effect on human development.	117 (65)	50 (27.8)	13 (7.2)
6. Food security and Biodiversity resource conservation does not have any Relation.	104 (57.8)	57 (31.7)	19 (10.5)
7. Maintaining habitat is fundamental to conserve species.	99 (55)	73 (40.6)	8 (4.4)
8. Loss of biodiversity in one area destroys the natural balance elsewhere.	67 (37.2)	87 (48.3)	26 (14.5)
9. Conservation means keeping and protecting a living environment.	136 (75.6)	36 (20)	8 (4.4)
10. Loss of biodiversity causes flooding, shortage of food, air pollution and global warming.	82 (45.6)	71 (39.4)	27 (15)

**Table 2.** Grade 12 students' (N=180) level of knowledge about biodiversity conservation (close ended questionnaire).

Question type	Respondent responses		
	Yes	No	Not sure
	F (%)	F (%)	F (%)
1. Biodiversity is the extent to which an ecosystem contains different species	128 (71.1)	49 (27.2)	3 (1.7)
2. Biodiversity uses as a human well-being	146 (81.1)	34 (18.9)	-
3. Biodiversity conservation refers to the practice of protecting and preserving the abundance and variety of all species.	160 (88.9)	19 (10.5)	1 (0.6)
4. Deforestation has negative impact on the biodiversity resources.	171 (95)	9 (5)	-
5. It is always better to repair an ecosystem rather than to replace it.	109 (60.6)	56 (31.1)	15 (8.3)
6. Climate change cannot control through biodiversity conservation	53 (29.4)	122 (67.8)	5 (2.8)
7. Protection of species and varieties of species will not support biodiversity.	75 (41.7)	103 (57.2)	2 (1.1)
8. Conservation of biodiversity resource has negative effect on human development.	81 (45)	99 (55)	-
9. Food security and Biodiversity resource conservation does not have any	80 (44.4)	93 (51.7)	7 (3.9)

Relation.			
10. Maintaining habitat is fundamental to conserve species.	117 (65)	63 (35)	-
11. Loss of biodiversity in one area destroys the natural balance elsewhere.	77 (42.8)	95 (52.8)	8 (4.4)
12. Conservation means keeping and protecting a living environment.	141 (78.3)	38 (21.1)	1 (0.6)
13. Loss of biodiversity causes flooding, shortage of food, air pollution and global warming.	79 (43.9)	90 (50)	11 (6.1)

As we can see the result from the above two tables (1 and 2) the same closed ended question were forwarded both grade 10 and 12 students. And when we see the understanding of the students about the knowledge and biodiversity conservation method grade 10 students much less understood as compared to grade 12 students. And as we have seen from table 1 only items number 1(56.1%), 2(72.8%), 10(55%) and 12 (75.6%) were responded correctly by majority of the students (above mastery level) from grade 10 but the rest question items were answered incorrectly by majority of the students from grade 10 (i.e. above 50% of the students answered incorrectly). But when we see grade 12 students ( table 2) almost all the items except item 11 and 13 were responded correctly by majority of the students ( i.e. above 50% of the students ). And this is in agreement with the study conducted in kebribeyah secondary and preparatory School, somali regional state, Ethiopia

most of the grade 10 student have no knowledge and awareness about Biodiversity and conservation method as compared to grade 12 students (Amanuel A., 2014). From this we can understand that still there is a big gap on student's general knowledge about biodiversity, its value, threatening factors, conservation method as well as relationship with environment and human wellbeing.

This is due to biodiversity conservation education which is currently given in school has not brought the expected change in student's knowledge particularly grade 10 as compared to grade 12 with regard to issue biodiversity and its conservation methods . So developing the knowledge of student's towards biodiversity conservation through education and different awareness creation mechanism is a key to conserve our biodiversity resources.

#### Open ended question result data

**Table 3.** Grade 10 students (N=180) written statements regarding their knowledge about Biodiversity Conservation.

Question item	Respondents response			
	CA	PA	WA	NA
	F (%)	F (%)	F (%)	F (%)
1. What is biodiversity and how to conserve it?	60 (33.3)	27 (15)	72 (40)	21 (11.7)
2. List threats of biodiversity in your area?	42 (23.3)	19 (10.6)	92 (51.1)	27 (15)
3. List the importance that we obtain from biodiversity?	73 (40.6)	29 (16.1)	69 (38.3)	9 (5)
4. What type of strategies used in biodiversity conservation?	33 (18.3)	31 (17.2)	97 (53.9)	19 (10.6)
5. List the site that used for conservation of biodiversity in your local area?	58 (32.2)	26 (14.4)	55 (30.6)	41 (22.8)
6. Please name at list five endemic plants and animals of Ethiopia?	89 (49.4)	18 (10)	59 (32.8)	14 (7.8)
7. What is your information about Ethiopian biodiversity institute before?	4 (2.2)	2 (1.1)	131 (72.8)	43 (23.9)
8. Describe your future plan with regard to biodiversity?	31 (17.2)	17 (9.4)	70 (39)	62 (34.4)

**Table 4.** Grade 12 students (N=180) written statements regarding their knowledge about Biodiversity Conservation

Question item	Respondents response			
	CA	PA	WA	NA
	F (%)	F (%)	F (%)	F (%)
1. What is biodiversity and how to conserve it?	109 (60.6)	24 (13.3)	40 (22.2)	7 (3.9)
2. List threats of biodiversity in your area?	89 (49.4)	26 (14.5)	48 (26.7)	17 (9.4)
3. List the importance that we obtain from biodiversity?	121 (67.2)	23 (12.8)	29 (16.1)	7 (3.9)
4. What type of strategies used in biodiversity conservation?	95 (52.8)	37 (20.6)	30 (16.6)	18 (10)
5. List the site that used for conservation of biodiversity in your local area?	78 (43.3)	49 (27.2)	42 (23.3)	11 (6.2)

6. Please name at list five endemic plants and animals of Ethiopia?	115 (63.9)	39 (21.6)	23 (12.8)	3 (1.7)
7. What is your information about Ethiopian biodiversity institute before?	24 (13.3)	17 (9.5)	77 (42.8)	62 (34.4)
8. Describe your future plan with regard to biodiversity?	91 (50.6)	27 (15)	47 (26.1)	15 (8.3)

**Note:** - *CA* = Correct Answered; *PA* = Partially Answered; *WA* = Wrong Answered; *NA* = Not Answered

From the above table 3 and 4 we can understand that the understanding level of Students at both grade level by providing the some type of question item. And when we see the understanding level of the student’s grade 10 students were not more understanding about the general concept and in all items that indicated in the table (i. e. below mastery level). But when we see grade 12 student’s level of understanding about the issue raised in the table they have more understanding than grade 10. Even if the students of grade twelve were better in their knowledge about biodiversity conservation still we can understand that there is a gap with in the knowledge about biodiversity. Focal group discussion which was stated by both grade level students supports the data that was gathered through close ended and open ended questionnaire which also used to evaluate students’ knowledge in different aspect about biodiversity conservation. In general from the above result of table 2 and 4 both in close ended and open ended question items respectively indicated that above 50% of the students’ from grade 12 responded correctly to almost all question items

of the close ended and open ended. Accordingly, the result implied that above 50% of the grade 12 students were above mastery level (i.e. above 50%).

**Assessment of Students’ Perception towards Biodiversity Conservation**

Result of assessment of students’ perception towards biodiversity conservation are summarized and presented in table 5 to 8.

The analysis of the items was made in terms of issues related with use of biodiversity resource, issue of responsibility, issue of reasons of biodiversity loss and issue of sustainable development about biodiversity conservation and the results of perception items are analyzed using frequency and percentage score.

Results of Assessment of grade 10 students’ perception towards biodiversity conservation particularly issue related to use and responsibility of biodiversity conservation are summarized and presented in table 5.

Table. 5. Grade 10 students’ (N=180) perception about biodiversity conservation (issue of related to use and responsibility of biodiversity conservation).

Question Items	Response		
	SA/A	SD/D	U
	F (%)	F (%)	F (%)
1. I believed that planting of trees use to protect climate change and biodiversity conservation	109(60.6)	68(37.8)	3(1.6)
2. I believe that the loss of biodiversity would affect our survival of life since Biodiversity is critical to human survival.	103(57.2)	43(23.9)	34(18.9)
3. I perceive that Forest clearance for agriculture or development is justifiable even if it affects Biodiversity resources.	19(10.6)	123(68.3)	38(21.1)
4. I think no need to bother about biodiversity resources as far as we secure our food from any source.	63(35)	92(51.1)	25(13.9)
5. I believe that Biodiversity resource loss does not have any impact on the socioeconomic and stability environment of Ethiopia.	27(15)	119(66.1)	34(18.9)
6. I agree that Biodiversity Conservation should mainly be the responsibility of the government rather than the local community.	51(28.3)	120(66.7)	9(5)
7. I believe that Students should not spend time to control biodiversity resources.	42(23.3)	127(70.6)	11(6.1)
8. According to my opinion individuals should be paid if they participate in biodiversity conservation activity.	21(11.7)	111(61.7)	48(26.6)
9. Conservation of biodiversity is not a matter that concerns me.	24(13.3)	117(65)	39(21.7)
10. As the students have little capacity to conserve their Biodiversity resources they should not be blamed.	77(42.8)	84(46.7)	19(10.5)
11. Once the biodiversity is exposed for reduction, it is wastage of time to conserve and Protect it.	68(37.8)	95(52.8)	17(9.4)
12. As citizens you have responsibility to participate voluntarily activities that are concerned with conservation of biodiversity.	115(63.9)	44(24.4)	21(11.7)

**Note:** - *SA/A* = strongly agree/agree, *SD/D* = strongly disagree/disagree, *U* = undecided

Planting trees have a great role in making stable the environment through taking in carbon dioxide from the atmosphere in the line of this, the result from

table 5, indicated 37.8% of students from grade 10 were found not perceived that the contribution of planting trees to biodiversity conservation and

climate change protection where as 1.6 % of the students were not certain on the issue. On the other hand 23.9% respondents from grade 10 believed that the loss of biodiversity cannot affect our quality of life and not critical to human survival and 18.9% of the students still not understand the value of biodiversity.

It is clear that forest resources are important to protect variety of species and to make stable the environment in line of this 10.6% of students from grade ten in the study agreed that agricultural productivity is impossible without clearing a forest where as 21.1% of students from grade ten were not certain about the issue. In addition 35% of the students also agreed that we do not bother about biodiversity resources as far as we secure our food from any source where as 13.9% of the students' undecided on these issue. Item 5 indicated that 15% of the students of grade ten believed that biodiversity loss do not have any impact on the socioeconomic and stability environment of Ethiopia. On the other hand 18.9% of students were not certain on the issue. Every citizens of the country have responsibility to care and conserve the biodiversity resources in the point view of this from the table 28.3% of the students from grade ten agreed that the biodiversity conservation is mainly the responsibility of the government while 5 % of students undecided about the responsibility of biodiversity conservation and it is observed that 23.3% of grade ten students in the study believed that the students should not spend time to control biodiversity resources. In addition to this again 11.7% of students from grade ten agreed that individuals should be paid if they participate in biodiversity conservation activity. 13.3% of the students from grade ten believed that the conservation of biodiversity resources do not concerns me and 42.8% of the students agreed that as the students have little capacity to conserve their Biodiversity resources they should not be blamed. 37.8 % of the students from grade ten agreed that

once the biodiversity is exposed for reduction, it is wastage of time to conserve and protect it. Furthermore 24.4% of students disagreed that as citizens you have responsibility to participate voluntarily and actively in activities that are concerned with conservation of biodiversity.

The result in table 5, item 1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 12 indicated that above 50% of the students had favorable perception regarding the use and responsibility of biodiversity conservation. Even if above 50% the students had favorable perception in the above mentioned item, but also above 30% of the students in question item 1, 4, 10, and 11 had shown unfavorable perception regarding the use and responsibility of biodiversity conservation and still some of the are undecided about the use and responsibility of biodiversity conservation This could be attributed to poor provision of biodiversity conservation awareness creation and education, the place given to biodiversity conservation education or other factors.

The basic knowledge and understanding of biodiversity; conservation of biodiversity resources and associated problems will help students to develop social value, strong feeling and responsibility for the environment and biodiversity conservation. Thus schools are responsible to address such an issue so that students will be knowledgeable of and concerned about the biodiversity conservation in which they live.

Results of Assessment of grade 10 students' perception towards biodiversity conservation particularly issue related to contribution of biodiversity conservation for sustainable development and reasons of loss of biodiversity are summarized and presented in table 6.

Table. 6. Grade 10 students' (N=180) perception about biodiversity conservation (issue of regarding sustainable development and reasons of loss of biodiversity).

Question items	Response		
	SA/A	SD/D	U
	F (%)	F (%)	F (%)
13. Human are superior to other species, for this reason they have the right to manipulate biodiversity to their will.	58(32.2)	99(55)	23(12.8)
14. As far as Charcoal is needed the community need not worry about Biodiversity resources.	47(26.1)	126(70)	7(3.9)
15. There is no harm in clearing forest land as far as the present generation satisfies its own need and as far as technology is progressing.	69(38.3)	97(53.9)	14(7.8)
16. As Ethiopia is rich in biodiversity resource there is no need to worry about biodiversity conservation.	76(42.2)	99(55)	5(2.8)
17. Conservation of biodiversity is far more important to care for the present generation than to think for the benefit of future generation.	54(30)	105(58.3)	21(11.7)
18. Biodiversity resources should be exhaustively utilized for human advantage at any cost.	66(36.7)	108(60)	6(3.3)
19. I believe that Biodiversity loss is not an environmental threat in Ethiopia.	80(44.5)	94(52.2)	6(3.3)

20. I do not want forest lands and parks to be regulated. People should be able to do what they want to do with this biodiversity resource.	64(35.6)	98(54.4)	18(10)
21. Biodiversity conservation does not have any economical value for our country.	59(32.8)	113(62.8)	8(4.4)
22. Different species are valued only because they are economically valued.	82(45.6)	88(48.9)	10(5.5)

Every species( starting from micro up to macro organism) where found in the world have equal right with the human being to live and the human being is not superior on other species in the line of this table 6, indicated 32.2% students from grade ten agreed that human are superior to other species, for this reason they have the right to manipulate biodiversity to their will and 26.1% of students from grade ten were also agreed that as far as Charcoal is needed the community need not worry about Biodiversity resources while 3.9% of students from grade ten undecided about this issue.

It is obvious that the present generation has responsibilities to transfer stable environment for the next generation but if the present generation do not give any attention to save and care our biodiversity resource, the future generation will be endanger in the line of this 38.3 % of students from grade ten agreed that there is no harm in clearing forest land as far as the present generation satisfies its own need and as far as technology is progressing and also 42.2% of student from grade ten believed that as Ethiopia is rich in biodiversity resource there is no need to worry about biodiversity conservation. And 30% of students from grade ten agreed that the conservation of biodiversity conservation is more important for the present generation than future generation.

Such perception can lead to exhaustive utilization of biodiversity resource without considering future development. For instance 36.7% of students of grade ten agreed that Biodiversity resources should be exhaustively utilized for human advantage at any cost and 44.5% also students from grade ten agreed that the Biodiversity loss is not an environmental threat in Ethiopia. Further 35.6% of the students agreed that i do not want forest lands and parks to be regulated. People should be able to do what they want to do with these biodiversity resources. On the other hand 32.8% of the respondents agreed that Biodiversity conservation do not have any

economical value for our country. Furthermore 45.6% of students believed that different species are valued only because they are economically valued.

The result showed that above 50% of the students from grade 10 had favorable perception to item number 13, 14, 15, 16, 17, 18, 19, 20, 21 and 22 with regard to issue of biodiversity conservation regarding its contribution for sustainable development and reasons of loss of biodiversity but also above 30% of the students had shown unfavorable perception towards items of number 13, 15, 16, 17, 18, 19, 20, 21 and 22 regarding sustainable development and loss of biodiversity.

This could either be due to level of maturity that grade ten students or having poor provision of biodiversity conservation education and awareness in schools and in the community or other related factors. Education as means to promote sustainability, students should be provided with information about their biodiversity, its utilization and conservation of biodiversity resources.

Increased knowledge and understanding of about biodiversity conservation and related issues help students to develop and promote perceptions of a conservation culture in biodiversity resources. Education should bring up citizen who can take care of the biodiversity resources and utilize resource wisely. It can also help students to develop sense of responsibility regarding use and management/conservation of biodiversity resource there by motivating them to actively and willingly participate in conservation of biodiversity programs. Results of Assessment of grade 12 students' perception towards biodiversity conservation particularly issue related to use and responsibility of biodiversity conservation are summarized and presented in table 7.

Table.7. Grade 12 students' (N=180) perception about biodiversity conservation (issue of regarding use and responsibility of biodiversity).

Question Items	Response		
	SA/A	SD/D	U
	F (%)	F (%)	F (%)
1. I believed that planting of trees use to protect climate change and biodiversity conservation	144(80)	26(14.4)	10(5.6)
2. I believe that the loss of biodiversity would affect our survival of life since Biodiversity is critical to human survival.	149(82.8)	19(10.6)	12(6.6)
3. I perceive that Forest clearance for agriculture or development is justifiable even if it affects Biodiversity resources.	27(15)	138(76.7)	15(8.3)
4. I think no need to bother about biodiversity resources as far as we secure our food from any source.	37(20.6)	141(78.3)	2(1.1)

5. I believe that Biodiversity resource loss does not have any impact on the socioeconomic and stability environment of Ethiopia.	49(27.2)	119(66.1)	12(6.7)
6. I agree that Biodiversity Conservation should mainly be the responsibility of the government rather than the local community.	21(11.7)	151(83.9)	8(4.4)
7. I believe that Students should not spend time to control biodiversity resources.	33(18.3)	137(76.1)	10(5.6)
8. According to my opinion individuals should be paid if they participate in biodiversity conservation activity.	53(29.4)	116(64.4)	11(6.2)
9. Conservation of biodiversity is not a matter that concerns me.	27(15)	138(76.7)	15(8.3)
10. As the students have little capacity to conserve their Biodiversity resources they should not be blamed.	77(42.8)	97(53.9)	6(3.3)
11. Once the biodiversity is exposed for reduction, it is wastage of time to conserve and Protect it.	59(32.8)	104(57.8)	17(6.4)
12. As citizens you have responsibility to participate voluntarily activities that are concerned with conservation of biodiversity.	152(84.4)	27(15)	1(0.6)

Plants can prepare their own food by the process of photosynthesis using light from the sun by the action of chlorophyll, water from the soil and carbon dioxide from the atmosphere. Carbon dioxide is one of the molecules that increase the world temperature now days. Therefore planting trees are very important to reduce the amount of carbon dioxide from the atmosphere and also uses to conserve biodiversity and to protect climate change in the line of this the above table showed that 14.4% of students from grade twelve disagreed that the contribution of planting trees to save different species and to reduce global warming where 5.6% of grade twelve students were not certain on the issue. On the other hand 10.6% and 15% of respondents from grade twelve believed that the loss of biodiversity cannot affect our quality of life and not critical to human survival and Forest clearance for agriculture or development is justifiable even if it affects Biodiversity resources respectively whereas 6.6 and 8.3% of the students respectively undecided about the issue. Furthermore 20.6% of agreed that we do not need to bother about biodiversity resources as far as we secure our food from any source where as 1.1% of students undecided on this issue.

It is clear that the loss of biodiversity can causes flooding, global warming, shortage of food, climate change, disease and lack of medicines and every citizens of the country have responsibility to care and conserve the biodiversity resources in the point view of this from result table 7, 27.2% and 11.7% of the students perceived Biodiversity resource loss does not have any impact on the socioeconomic and stability environment of Ethiopia and Biodiversity Conservation should mainly be the responsibility of the government rather than the local community respectively where as 6.7% and 4.4% of the students' undecided about the issue respectively. In addition 18.3% and 29.4% of the students believed that Students should not spend time to control biodiversity resources and individuals should be paid if they participate in biodiversity conservation activity where as 5.6% and 6.2% of the students' undecided about the issue respectively. On the other hand 15% of students from grade twelve believed that the conservation of biodiversity resources is not

a matter that concerns me. 42.8% and 32.8% of students from grade ten agreed that as the students have little capacity to conserve their Biodiversity resources they should not be blamed and once the biodiversity is exposed for reduction, it is wastage of time to conserve and protect it. Furthermore 15% of students disagreed that as citizens you have responsibility to participate voluntarily and actively in activities that are concerned with conservation of biodiversity.

The result in table 7 above 70% of the students had favorable perception to items number 1, 2, 3, 4, 6, 7, 9, and 12 regarding the use and responsibility of biodiversity conservation. This implying that as the grade level increase perception towards biodiversity also increases. As far as the use conservation of biodiversity resources is concerned the assessment of student text book indicated that the diversified uses conservation of biodiversity resource were included widely in grade twelve .Clear understanding of the use of conservation biodiversity resource is instrumental to develop strong perception of concern for the biodiversity conservation and for actively participating in protecting and improving the biodiversity resources. To this end education is an important tool and schools are one of the places and should provide students with information about the use and responsibility of biodiversity conservation. With regard to issue of the use and responsibility the result in terms of their grade level showed that grade twelve students had more favorable perception than students of grade ten. This implying that the learning experience and increasing grade level have its own contribution on the development of student's perception towards biodiversity conservation.

Results of Assessment of grade 12 students' perception towards biodiversity conservation particularly issue related to contribution of biodiversity conservation for sustainable development and reasons of loss of biodiversity are summarized and presented in table 8.

Table. 8. Grade 12 students' (N=180) perception about biodiversity conservation (issue of regarding sustainable development and reasons of loss of biodiversity).

Question items	Response		
	SA/A	SD/D	U
	F (%)	F (%)	F (%)
13. Human are superior to other species, for this reason they have the right to manipulate biodiversity to their will.	38(21.1)	133(73.9)	9(5)
14. As far as Charcoal is needed the community need not worry about Biodiversity resources.	36(20)	140(77.8)	4(2.2)
15. There is no harm in clearing forest land as far as the present generation satisfies its own need and as far as technology is progressing.	37(20.6)	141(78.3)	2(1.1)
16. As Ethiopia is rich in biodiversity resource there is no need to worry about biodiversity conservation.	21(11.7)	152(84.4)	7(3.9)
17. Conservation of biodiversity is far more important to care for the present generation than to think for the benefit of future generation.	48(26.7)	124(68.9)	8(4.4)
18. Biodiversity resources should be exhaustively utilized for human advantage at any cost.	27(15)	149(82.8)	4(2.2)
19. I believe that Biodiversity loss is not an environmental threat in Ethiopia.	33(18.3)	143(79.5)	4(2.2)
20. I do not want forest lands and parks to be regulated. People should be able to do what they want to do with this biodiversity resource.	17(9.4)	160(88.9)	3(1.7)
21. Biodiversity conservation does not have any economical value for our country.	15(8.3)	164(91.1)	1(0.6)
22. Different species are valued only because they are economically valued.	78(43.4)	94(52.2)	8(4.4)

It is obvious that all living species have the right to co-exist with human on earth and humans have no right to cause the extinction or to diminish the quality of life of organism but the result in table 8 it was observed that 21.1% of the students from grade 12 agreed that Human are superior to other species, for this reason they have the right to manipulate biodiversity to their will. In addition to this again 20% of the students agreed that as far as Charcoal is needed the community need not worry about Biodiversity resources. 20.6% and 11.7% of the students were also perceived that there is no harm in clearing forest land as far as the present generation satisfies its own need and as far as technology is progressing and as Ethiopia is rich in biodiversity resource there is no need to worry about biodiversity conservation respectively.

According to Mazengia Shimelis (2010) Sustainable development aims at continuously improving the people's lives through rational exploitation and optimal utilization of natural resources on one hand and maintaining environmental quality and ecological balance on the other hand so that there may be continuous supply of natural resources to the present and future generation without harming the environment in the line of this 26.7% and 15% of students agreed that Conservation of biodiversity is far more important to care for the present generation than to think for the benefit of future generation and Biodiversity resources should be exhaustively utilized for human advantage at any cost respectively where as 4.4% and 2.2% of the students undecided about the issue respectively. 18.3% of the students believed that Biodiversity loss is not an environmental threat in Ethiopia.

National parks and forest land have great role in saving and conserving the biodiversity resources and it is also obvious that the biodiversity resources have great economical value in one country like as a source of food and medicines, to control flooding and global warming and also it uses to maintain stable environment regarding this point of view the result in table 8, indicated that 9.4% of students from grade agreed that i do not want forest lands and parks to be regulated. People should be able to do what they want to do with these biodiversity resources and 8.3% of the students also perceived that Biodiversity conservation do not have any economical value for our country. In addition to this 43.4% of the students believed that different species are valued only because they are economically valued where as 4.4% of the students were not certain about the issue.

The result in table 8 showed that above 70% of the students from grade 12 had favorable perception in question item 13, 14, 15, 16, 18, 19, 20, and 21 regarding the reasons of loss of biodiversity and contribution of biodiversity conservation for sustainable development and this implied that students from grade 12 showed more favorable perception with regard to issue of biodiversity conservation regarding reasons of loss of biodiversity and contribution of biodiversity conservation for sustainable development. According Trong Trai *et al.*, (2001) study indicated that having low perception of one society has negative impact in the conservation of biodiversity resource. Therefore the above results indicated that even if above 50% and 70% of the students from grade 10 and 12 had shown favorable perception towards biodiversity conservation respectively but if only and only all the society have good perception towards biodiversity

conservation to protect our biodiversity resources perfectly.

As resources have diversified values, the rational use of biodiversity resource is instrumental for socio-economic and political wellbeing of a society. Proper management and conservation of biodiversity resources is a means to maintain the balance between natural diversity and human sustainable living. As human beings require biodiversity resources in many ways the rational use of resource is vital for human development. Unwise resources management can lead to resource degradation affecting human life. As sustainability requires ecological sustainability which in turn depend on conservation of biodiversity resources education should provide students with the basic information about the biodiversity there by helping them to have knowledge and develop skill to take care of the biodiversity resources and to live in harmony with it.

## CONCLUSION

The review of different literature in this study indicated the diversified value of biodiversity resource for human being. Furthermore biodiversity resource conservation is seen as an important tool for sustainable development and essential issue of biodiversity education. Mainly the loss of biodiversity practiced due to lack of knowledge and perception of the society towards biodiversity conservation. To solve the problem biodiversity conservation education can play an important role in bringing change in the knowledge and perception of student's. As far as the objectives are concerned biodiversity conservation education is meant to help individuals and social groups acquire knowledge of and sensitivity to the total environment and its allied problems. Further to help individuals and social groups acquire social value, strong feelings of concern for the biodiversity and the motivation for actively participating in its protection and improvement. Above 50% of the students of grade 12 were found above mastery level knowledge (i.e. 50%) and also above 70% of the students had shown favorable perception regarding biodiversity conservation respectively where as above 50% of students of grade 10 were found below mastery level of knowledge and above 30% of the students of grade 10 had shown unfavorable perception towards biodiversity conservation, although above 50% had shown favorable perception regarding biodiversity conservation. Therefore it can be concluded that the currently given education has not brought the expected change in knowledge and perceptions among students of grade 10 towards biodiversity conservation in the study area due to many problems.

### Recommendations

The study has proved that there were variation in knowledge and perception among the study groups, where as grade twelve students were found better

both in their knowledge and perception than students of grade ten. The observed difference could be attributed to different factors and thus the following points are put as recommendations of the study:-

Gaining efficient knowledge about biodiversity conservation and developing positive perception towards the biodiversity conservation is possible through effective biodiversity conservation Education. Thus continued effort should be conducted to raise student's level of knowledge and perception by properly addressing biodiversity conservation issues through effective provision of biodiversity conservation education in schools.

Schools should intensively address biodiversity conservation issues and should mobilize and participate students so as to be knowledgeable of and develop a strong perception towards biodiversity conservation. This could be done by organizing biodiversity clubs, assigning and celebrating biodiversity conservation weeks, facilitating a biodiversity conservation education field trip so that students can have real biodiversity conservation experience and transform their theoretical knowledge in to behavior easily, organizing seminars and workshops on biodiversity issues, sharing and adapting important biodiversity conservation education experience from other schools or other possible mechanisms.

Further investigation should be conducted to find out:-

Whether or not subject teachers are given training on how to integrate biodiversity conservation Education in their subject area.

Whether or not the actual teaching and learning process and methods employed in schools properly address issue of Biodiversity Conservation.

The existing constraints that influence the provision of Biodiversity Conservation Education.

### Conflict of interest

The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper.

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## VARIABLE SALINITY TOLERANCE IN ANABAENA SP. BHUAR002 THROUGH REGULATION OF ION UPTAKE AND PRODUCTION OF OSMOPROTECTANT

Aparna Rai\*

Department of Botany, Banaras Hindu University (BHU), Varanasi 221005, India

Email: [aparna.raii82@gmail.com](mailto:aparna.raii82@gmail.com)

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**Abstract:** Filamentous, heterocyst-forming, diazotrophic cyanobacterium *Anabaena* sp. BHUAR002 was isolated from usar (saline) land near Banaras Hindu University campus, and grown routinely on Allen Arnon medium. The growth of cyanobacterium was measured at various concentrations (upto 1000 mM) of different salt combinations, NaCl, NaCl+Na<sub>2</sub>CO<sub>3</sub> (1:1) and NaCl+Na<sub>2</sub>SO<sub>4</sub> (1:1) and found that the cyanobacterium tolerated the salinity of 500 mM NaCl, 700 mM NaCl+Na<sub>2</sub>CO<sub>3</sub> and 1000 mM NaCl+Na<sub>2</sub>SO<sub>4</sub>, indicating that elevated carbonate and sulphate support the growth of cyanobacterium under salinity and increase the tolerance range. Natural abundance <sup>13</sup>C-NMR spectra chemical shifts showed sucrose as the osmoticum synthesized in NaCl and NaCl+Na<sub>2</sub>CO<sub>3</sub> (1:1). However, synthesis of sucrose was not found in case of NaCl+Na<sub>2</sub>SO<sub>4</sub> (1:1). Intracellular Na<sup>+</sup> concentration increases under different salt concentrations as compared to control. K<sup>+</sup> concentration also increases with increase of different salt concentration as compared to control is also an indication of acclimatization against salt stress; this type of ionic ratio was found in all three salt stress conditions. Intracellular Cl<sup>-</sup> concentration was found minimum in case of NaCl+Na<sub>2</sub>SO<sub>4</sub> as compared to NaCl and NaCl+Na<sub>2</sub>CO<sub>3</sub> incubated cells.

**Keywords:** Intracellular ion concentration, Osmotic, Salinity, Tolerance range

### INTRODUCTION

As organisms originated millions of years ago (Brock 1973) have passed successfully through several conditions generated by the environment on the earth. Cyanobacteria shows diverse stress response and offer an excellent prospect for conducting studies particularly the ability of heterocystous cyanobacteria to tolerate stresses as deficiency of nutrient, salinity and temperature (Apte et al. 1987; Aparna Rai 2015). Salt tolerance have been shown by many cyanobacteria (Thomas and Apte 1984), salt tolerance enhanced by presence of certain nitrogenous compounds in the growth medium (Reddy et al. 1989) and all treatments which inhibit Na<sup>+</sup> influx (such as alkaline pH, K<sup>+</sup> above 25 mM, NO<sub>3</sub><sup>-</sup>, NH<sub>4</sub><sup>+</sup>) have shown an enhancement in the salt tolerance for brackish-water and also for the freshwater cyanobacterium (Apte et al. 1987). It has also found that cyanobacteria get acclimatizes to salt stress by ion regulation and osmoprotectants formation (Pade and Hagemann 2014). Similar studies were conducted on plants where it shows that plant adopts SOS pathway for regulation of ion uptake (Gupta and Huang 2014) during salinity stress. Salt adaptation by freshwater cyanobacteria is composed of several mechanisms. Studies on the N<sub>2</sub> fixing cyanobacterium *Nostoc muscorum* grown at high NaCl concentrations revealed stimulation of photosynthetic activity and sucrose accumulation (E Blumwald and Telor 1982), photoautotrophic nitrogen-fixing cyanobacteria in general exhibits considerable tolerance to salt or osmotic stress (Thomas and Apte 1984). One of the

cyanobacterial approaches for the problem of saline soil was proposed in the 1950's, where in cyanobacteria fixing nitrogen from the atmosphere naturally were employed for the reclamation of saline/alkaline 'usar' soil typical of certain North Indian States (Singh 1950, 1961).

### MATERIAL AND METHOD

**Isolation and purification of cyanobacteria-** Surface soils (3-4 inch of the upper layer) from the usar fields of the nearby locality were collected and brought to the laboratory. Soil samples were ground to the powder and mixed. A known amount of soil samples (5 g) was taken in an autoclaved tube and added sterile double distilled water (5 mL). The samples were mixed thoroughly by shaking and allowed to stand for half an hour. After settling the soil particles, the supernatant was used to inoculate in combined nitrogen-free agar plates of Allen Arnon (AA) medium (Allen and Arnon 1955) containing different concentrations of NaCl (100mM, 300mM, 500mM, 700mM and 1000 mM) to isolate nitrogen-fixing cyanobacterial forms. Plates were placed in a culture room set at 28 ± 1°C, illuminated with daylight fluorescent lamps at photon fluence rate of 95 μmol m<sup>-2</sup> s<sup>-1</sup> under 16: 8 h light-dark period to allow the growth of cyanobacteria. After a week cyanobacterial colonies appeared on the plates of 100 – 500 mM NaCl. However, no colonies appeared at 700 and 1000 mM NaCl containing plates. Since our interest was to isolate salt tolerant cyanobacterial forms, therefore, individual colonies appearing on plates of 500 mM NaCl were picked up

\*Corresponding Author

and subcultured in liquid medium using standard microbiological techniques. A single strain of cyanobacterium was obtained in unialgal culture condition. Morphological characters (filament sheath, length, width, shape and size of vegetative cells and heterocysts, etc.) and keys of Desikachary (Desikachary 1959) were used to identify the cyanobacterial strain as *Anabaena* sp. Further 16S ribosomal RNA partial sequencing of isolated DNA and the sequences were submitted in NCBI where it is identified as new species named *Anabaena* sp. BHUAR002 (Accession no. bankit1353506 HM235817) (A Rai and Rai 2011). Axenic nature of isolated cyanobacteria was ensured periodically by plating the diluted cultures on caseinate-glucose agar AA medium (free of combined nitrogen) supplemented with (w/v) casamino acids (0.05%), glucose (0.5%) and agar (1%), and incubated under standard growth conditions. To eliminate bacterial contamination, if any, clean microcolonies were transferred to AA liquid medium and allowed to grow under standard growth conditions.

**Design of simulated saline condition-** Salt of 100mM, 300mM, 500mM, 700mM and 1000mM concentrations of NaCl and salt mixture of these concentrations using combinations of either two salts NaCl+Na<sub>2</sub>CO<sub>3</sub> and NaCl+Na<sub>2</sub>SO<sub>4</sub>, mixed at a 1:1 molar ratio (Yang et al. 2007), for the salt treatment concentrations referred to the total salt concentrations of NaCl + Na<sub>2</sub>CO<sub>3</sub> or NaCl + Na<sub>2</sub>SO<sub>4</sub>. Therefore, in 100mM solution, a mixture of 50mM NaCl and 50mM Na<sub>2</sub>CO<sub>3</sub> would result in total ion concentrations of 150mM Na+50mM Cl+50mM CO<sub>3</sub>. For 100mM NaCl+Na<sub>2</sub>SO<sub>4</sub> solution, a mixture of 50mM NaCl and 50mM Na<sub>2</sub>SO<sub>4</sub> result in total ion concentrations of 150mM Na+50mM Cl +50mM SO<sub>4</sub> like this the five concentration treatments for each pair were applied.

**Stress treatment-** Exponentially growing cyanobacterial cells were subjected to stress treatment by inoculating it at 20:1 (20 is the medium and 1 is inoculum) ratio on different concentrations of salts, different combinations of salts and also in control.

**Growth estimation -** Salt tolerance in cyanobacteria has been studied mainly for their ability to tolerate different salt levels. Very few studies describe their ability to tolerate or grow in different salts and /or their combinations. We measured the growth of cyanobacteria at different salt combinations and concentrations. Exponentially growing acyanobacterial strain of *Anabaena* sp. BHUAR002 were inoculated in combined N-free sterile AA medium supplemented with NaCl, NaCl + Na<sub>2</sub>CO<sub>3</sub> (1:1) and NaCl + Na<sub>2</sub>SO<sub>4</sub> (1:1) (Yang et al. 2007) to get final salt concentrations of 100 mM, 300 mM, 500 mM, 700 mM and 1000 mM. Growth was measured by recording the absorbance of the cultures daily at 650 nm in a spectrophotometer (Milton Roy, Spectronic 20).

**Measurement of osmoticum** – In an attempt to find out the osmoticum synthesized by cyanobacteria to counteract the effect of salts, cells of *Anabaena* sp. BHUAR002 were incubated in Allen Arnon medium with different salt concentrations of NaCl, NaCl + Na<sub>2</sub>CO<sub>3</sub> (1:1) and NaCl + Na<sub>2</sub>SO<sub>4</sub> (1:1) under standard growth conditions. Sampling was done at regular intervals for 96 h and estimated the osmoprotectants such as sucrose, trehalose and glycine betaine spectrophotometrically. Sucrose was measured as described by (Handel 1968), trehalose as (Lillie and Pringle 1980) and glycine betaine as (Wall et al. 1960) at different salt combinations and concentrations.

**Ion estimation:** Exponentially growing cells of *Anabaena* sp. BHUAR002 was incubated at different concentrations of salts of different combinations under standard growth conditions. After 24 h, the cells were collected by centrifugation and analyzed the intracellular ion content employing atomic absorption spectrometer (Association of Official Analytical Chemists 1984; Rodkey and JR 1963).

## OBSERVATION AND RESULT

**Growth estimation-** *Anabaena* sp. BHUAR002, tested grew well in AA medium. However, the growth was maximal when the medium contained 100 mM NaCl; growth (yield) increased by 13% over the control. 300 mM NaCl conc, also stimulated the growth as well as yield, it is 8% over the control. Cyanobacterial strain tolerated the salinity of 500 mM NaCl, but with reduced yield. Yield reduction at 500 mM NaCl was observed 15% to that of control, however, cyanobacterium could not tolerate NaCl concentrations of 700 mM and beyond. This indicates that *Anabaena* sp. BHUAR002 is salt tolerant upto 500mM in case of NaCl as a salt.

To find out the growth of cyanobacterium at elevated CO<sub>2</sub> concentration, NaCl+Na<sub>2</sub>CO<sub>3</sub> increased the growth of cyanobacterial strain at low salt concentrations of 100 and 300 mM NaCl+Na<sub>2</sub>CO<sub>3</sub>. Salt concentration of 100 mM increased the yield of *Anabaena* sp. BHUAR002 by 93%, over the control. Growth stimulation of cyanobacterium was also evident at 500 mM NaCl+Na<sub>2</sub>CO<sub>3</sub>, and the yield was found to be increased by 19%, over the control. cyanobacterium was able to sustain even at salt concentration of 700 mM NaCl+Na<sub>2</sub>CO<sub>3</sub> with reduced growth rate and yield. Growth at 700 mM salt concentration was identical to that of control. The data thus indicated that elevated carbonate supported the growth of *Anabaena* sp. BHUAR002 strain under salinity and increased the tolerance range. However, 1000 mM NaCl+Na<sub>2</sub>CO<sub>3</sub> inhibited the cyanobacterial growth completely. Further it was found that with the salt combination of NaCl+Na<sub>2</sub>SO<sub>4</sub> the yield was increased at lower salt concentration of 100 and 300 mM. Cyanobacterium was found to grow even at 1000 mM salt

concentration, although the yield was reduced. This indicated that presence of  $\text{SO}_4$  in the saline environment not only protected the cyanobacterial cells from salt toxicity, albeit increased the range of salt tolerance to 1000 mM (Figure-1, Table-1).

**Osmoticum** - Sucrose and trehalose were synthesized maximum at salt concentration of 200 mM of NaCl and NaCl+Na<sub>2</sub>CO<sub>3</sub> at 24 h of incubation (Figure-2).

Since methods used for estimation of sucrose and trehalose was based on anthrone test, we confirmed the compounds by <sup>13</sup>C-NMR using standard sucrose, trehalose and glycine betaine. Natural abundance <sup>13</sup>C-NMR spectra chemical shifts coincided with that of sucrose standard and not with trehalose and glycine betaine proving sucrose as the osmoticum synthesized by *Anabaena* sp. BHUAR002 (Figure-3, Figure-4 and Table -4).

Presence of NaCl in the nutrient solution induces the synthesis of sucrose and trehalose, both were found 165.18% and 101.68% respectively when compared to control at 200 mM NaCl. Further, when *Anabaena* sp. BHUAR002 exposed to the different concentrations of NaCl+Na<sub>2</sub>CO<sub>3</sub>, sucrose and trehalose synthesized maximum at 200 mM salt concentration and both were found to be 130.63% and 24.35% respectively to that of control. Presence of 200 mM of NaCl+ Na<sub>2</sub>SO<sub>4</sub> does not induce the synthesis of sucrose (Table-2). It was further confirmed by <sup>13</sup>C-NMR spectra which do not show any chemical shifts towards the standard of sucrose in case of NaCl+Na<sub>2</sub>SO<sub>4</sub> (Table-4).

**Intracellular ion concentrations-** Increasing salt (NaCl, NaCl+Na<sub>2</sub>CO<sub>3</sub>, NaCl+Na<sub>2</sub>SO<sub>4</sub>) concentration in the medium, increases the intracellular Na<sup>+</sup> content accordingly. Intracellular K<sup>+</sup> ion at various selected concentrations of NaCl have lesser values in comparison to control but the K<sup>+</sup> content have shown an increasing trend from 100 to 1000 mM concentration of NaCl. It was observed that upto 700 mM NaCl + Na<sub>2</sub>CO<sub>3</sub> intracellular level of potassium ion gradually increases but at 1000 mM sudden drop was observed. Organism growth was also arrested at 1000 mM NaCl + Na<sub>2</sub>CO<sub>3</sub> concentration. Intracellular K<sup>+</sup> content under NaCl + Na<sub>2</sub>SO<sub>4</sub> increases with the increase in concentration of salt from 100 mM to 1000 mM. Increasing level of intracellular potassium ion content upto 1000 mM indicated and supported the growth of organism upto 1000 Mm.

Salinity increases the calcium content in *Anabaena* sp. BHUAR002 upto 1000 mM NaCl concentrations. On observing the pattern of intracellular Ca<sup>2+</sup> in the case of NaCl + Na<sub>2</sub>CO<sub>3</sub>, it was found that Ca<sup>2+</sup> concentration gradually increases from 100 to 700 and decreases at 1000 mM. This indicated that increase of intracellular calcium content from 100 to 700 mM NaCl + Na<sub>2</sub>CO<sub>3</sub> help organism to survive and tolerate such a high degree of salt stress and 1000 mM was found unfavorable for organism growth. As similar to intracellular potassium ion

content, intracellular calcium ion content was also found increasing upto 1000 mM NaCl + Na<sub>2</sub>SO<sub>4</sub> salt stress. So it is concluded that intracellular calcium ion content also helps the cyanobacterial cells to tolerate NaCl + Na<sub>2</sub>SO<sub>4</sub> upto 1000 mM. (Table-3)

Intracellular chloride ion content increases with increase in NaCl concentration from control to 1000 mM NaCl. Accumulation of Intracellular chloride ion content under NaCl + Na<sub>2</sub>CO<sub>3</sub> stress and NaCl + Na<sub>2</sub>SO<sub>4</sub> stress was similar to NaCl stress i.e., level of Intracellular chloride ion content increases with increasing concentration of NaCl + Na<sub>2</sub>SO<sub>4</sub> but its value was low as compared to NaCl and NaCl + Na<sub>2</sub>CO<sub>3</sub>. (Table-3)

## DISCUSSION

The data obtained from this investigation strongly suggest that the very rapid NaCl entry into the cell triggers the adaptive response of the cyanobacteria to salt and that both organic and osmoregulatory mechanism are involved in this process. Osmoregulation can be accounted by the intracellular concentrations of sucrose, K<sup>+</sup>, and residual Na<sup>+</sup> ions. For 200mM NaCl grown cells, there is a relatively rapid accumulation of sucrose followed by a more gradual accumulation of K<sup>+</sup> suggesting that the initial osmoregulatory response of the cells is a 'compatible' solute, i.e. a solute which is not disruptive of macromolecular interaction (Eduardo Blumwald et al. 1983). However, when salt is NaCl+Na<sub>2</sub>SO<sub>4</sub> there is a very small accumulation of sucrose; it means some other regulatory mechanisms also operate regarding the salt tolerance under sulphate supplement combined salt stress of NaCl+Na<sub>2</sub>SO<sub>4</sub>.

The growth of cyanobacteria in NaCl salt stress found up to 500mM, but when carbonate was given as a supplement with NaCl, the tolerance level was found to be increased upto 700mM NaCl+Na<sub>2</sub>CO<sub>3</sub> concentrations. However, when salt was NaCl+Na<sub>2</sub>SO<sub>4</sub>, the growth of cyanobacteria became luxuriant even at 1000mM concentration. These results show that carbonate and sulphate both are growth inducer in *Anabaena* sp. BHUAR002. Primarily carbonate gives some sort of tolerance (up to 700mM), one reason behind this is CO<sub>2</sub> concentration, which increases the rate of photosynthesis and organism grow properly. However, when we think about the factors responsible for increased salt tolerance in cyanobacteria, two aspects can be considered; one is that the intracellular ions play a major role for the survival of organism at higher salt concentration and the second is that the formation of an osmoprotectant. In case of NaCl+Na<sub>2</sub>CO<sub>3</sub>, osmoprotectant play a major role in cyanobacterial growth under high salt concentration of 700mM. In *Anabaena* sp. BHUAR002 in comparison to intracellular ion concentrations. However, in case of NaCl+Na<sub>2</sub>SO<sub>4</sub>, intracellular ion concentration and osmoprotectant

both play certain role for the survival of organism at high salt concentration(1000mM) but some other mechanism is also involved that helps organism to survive so luxuriantly under high salt concentration which is not known.As mentioned elsewhere in result section that osmoprotectant formed in NaCl and NaCl+Na<sub>2</sub>CO<sub>3</sub> is sucrose but when salt is

NaCl+Na<sub>2</sub>SO<sub>4</sub> sucrose is not formed. Same way K<sup>+</sup> accumulation is also found less in case of NaCl+Na<sub>2</sub>SO<sub>4</sub> as compared to NaCl and NaCl+Na<sub>2</sub>CO<sub>3</sub>.  
Acknowledgment- Thanks are due to Prof. A. K. Rai, Department Of Botany, Banaras Hindu University, Varanasi, India, for suggestions.

**Table 1.** Showing percentage increase/decrease yield of *Anabaena* sp. BHUAR002 under different concentrations and combinations of salts. (AA represent Allen Arnon medium as control).

Conc (mM)	Yield (%)		
	NaCl (mM)	NaCl+Na <sub>2</sub> CO <sub>3</sub> (1:1)	NaCl+Na <sub>2</sub> SO <sub>4</sub> (1:1)
Control(AA)	100	100	100
100	112.5	193.1	108.386
300	108.3	128.7	119.7
500	85.4	118.81	101.8
700	14.6	98.02	76.64
1000	2.1	8.912	76.64

**Table 2.** Percentage increase/decrease in the content of sucrose and trehalose in *Anabaena* sp. BHUAR001 and *Anabaena* sp. BHUAR002 with varying concentrations of salts.

% increase/decrease of osmoprotectant				
Salt concentration	<i>Anabaena</i> sp. BHUAR002			
	Sucrose	Trehalose	Sucrose	Trehalose
NaCl			(NaCl+Na <sub>2</sub> CO <sub>3</sub> )	
Control	100	100	100	100
100 mM	212.74	160.22	209.5	86.6
200 mM	265.18	201.68	230.63	124.35
300 mM	246.69	167.05	221.97	103.35
400 mM	147.08	142.97	186.29	98.52
500 mM	98.54	134.95	132.21	98.32

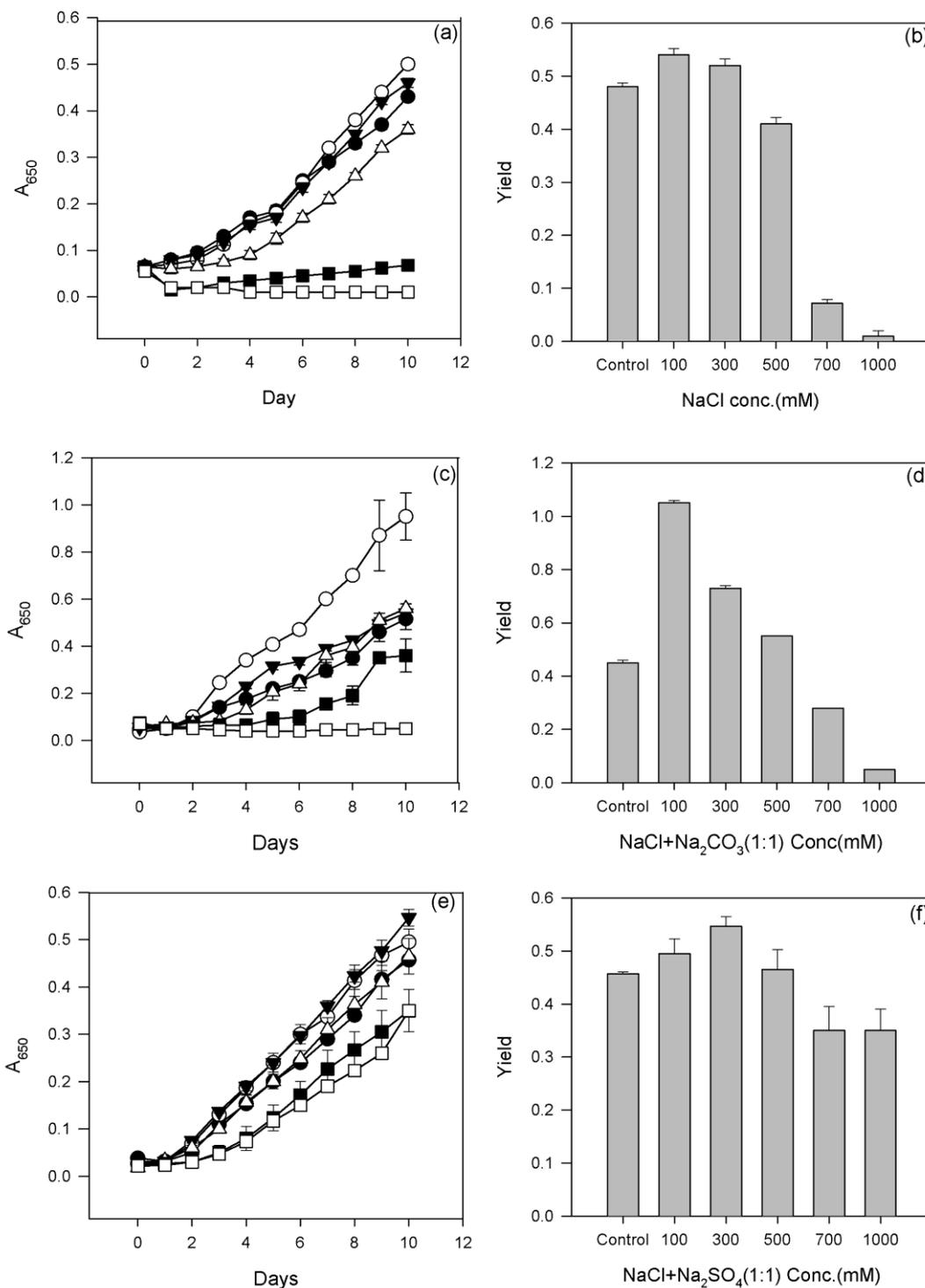
**Table 3.** Showing different intracellular cations and anion in *Anabaena* sp. BHUAR002 under different concentrations and combinations of salts.

Salt (mM)	Intracellular ion content (mmol.µg chl-1) of <i>Anabaena</i> sp. BHUAR002			
	Na <sup>+</sup>	K <sup>+</sup>	Ca <sup>2+</sup>	Cl <sup>-</sup>
NaCl				
Control	0.95	2.77	0.48	1.53
100	6.53	1.64	0.71	7.20
300	25.81	1.91	1.68	25.67
500	27.73	1.17	1.72	27.53
700	43.30	0.70	1.37	41.67
1000	55.14	0.09	0.09	54.00

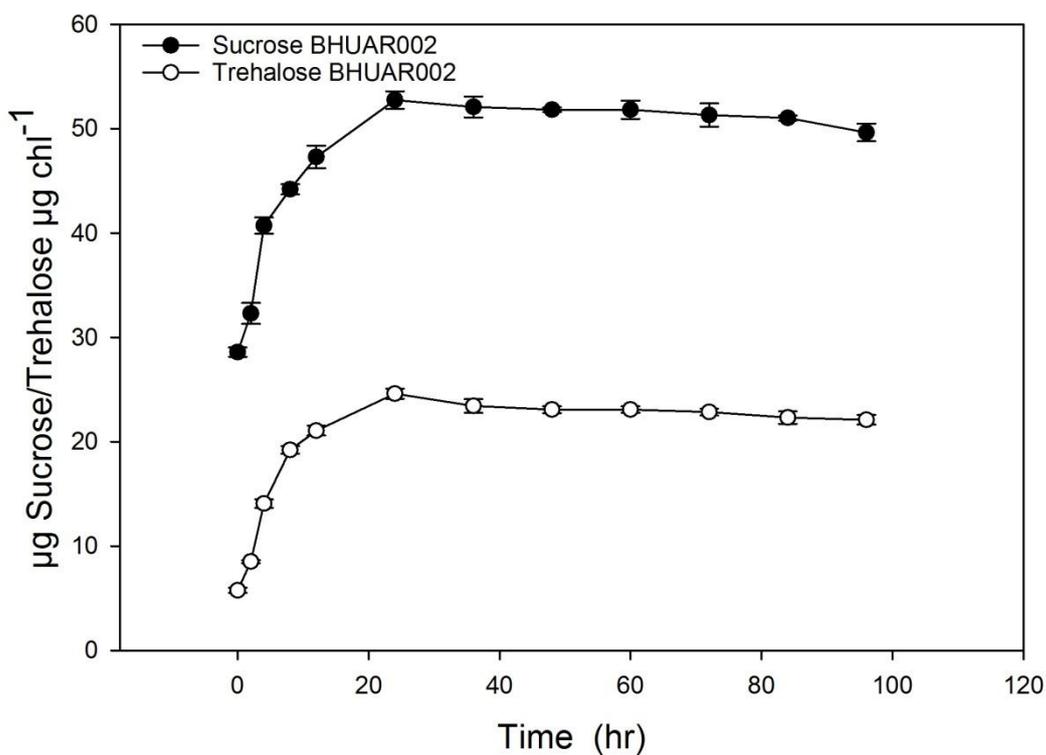
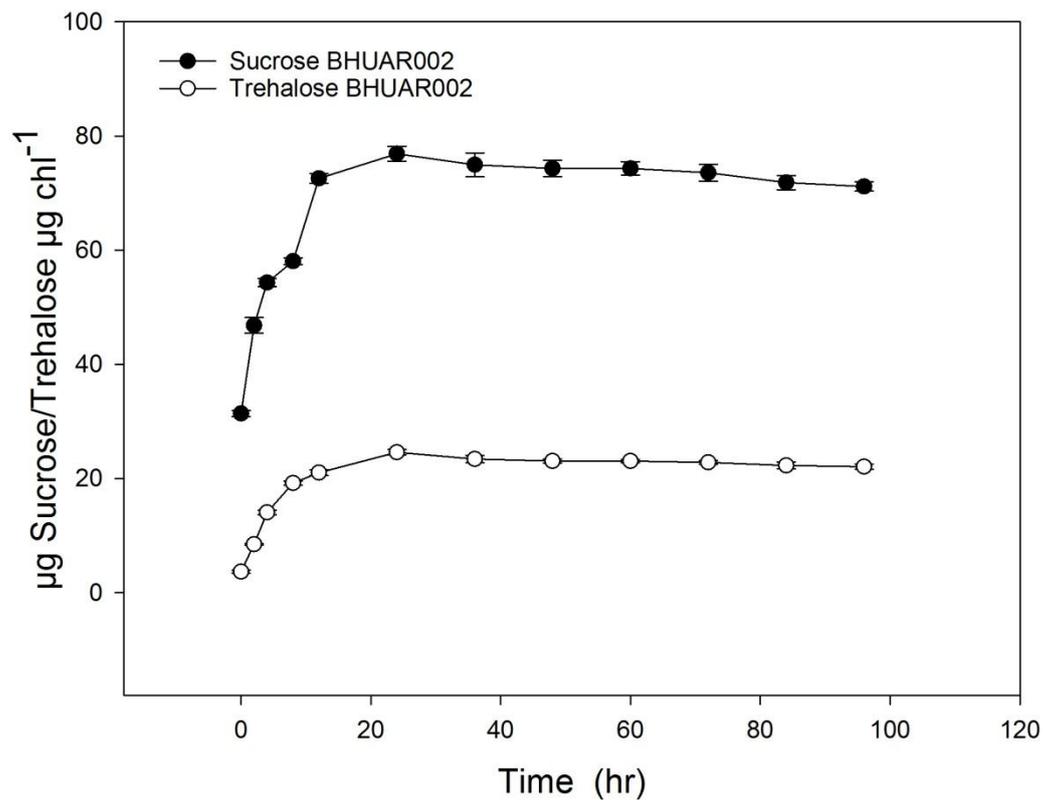
<b>NaCl+Na<sub>2</sub>CO<sub>3</sub></b>				
100	6.39	1.75	0.84	7.20
300	25.55	2.19	1.39	26.33
500	26.90	1.95	1.47	27.33
700	40.80	2.10	1.50	40.83
1000	51.00	1.27	1.20	53.33
<b>NaCl+Na<sub>2</sub>SO<sub>4</sub></b>				
100	5.06	0.56	0.55	1.68
300	13.30	0.90	1.37	4.42
500	37.09	1.21	2.04	9.82
700	52.97	1.92	2.44	11.01
1000	94.37	1.96	5.14	23.37

**Table 4.** <sup>13</sup>C-NMR spectrum of sucrose, trehalose and glycine betaine (standards) and its chemical shifts (δppm) in *Anabaenasp.* BHUAR002 at different salt combinations.

Std Sucrose	StdTrehalose	Std Glycine betaine	Control	NaCl	NaCl +Na <sub>2</sub> CO <sub>3</sub> (1:1)	NaCl +Na <sub>2</sub> SO <sub>4</sub> (1:1)
			<i>Anabaenasp</i> BHUAR002			
49.9	49.9	49.9	49.9	49.9	49.9	49.9
		54.853				
61.084						
	61.521					
		64.521				
70.166						
	70.669					
	73.084					
73.323						
	73.504					
73.529				73.529		
74.946					74.946	
77.361						
82.298				82.298		
93.086						
	94.149					
104.6				104.6		
		167.83				



**Figure 1.0.** (a, b) Showing growth and yield of *Anabaena* sp. BHUAR002 at 10<sup>th</sup> day of growth under different NaCl concentrations respectively likewise (c, d) showing growth and yield (at 10<sup>th</sup> day) respectively when salt is NaCl+Na<sub>2</sub>CO<sub>3</sub> (1:1), and (e, f) also showing growth and yield (at 10<sup>th</sup> day) respectively of *Anabaena* sp. BHUAR002 when salt is NaCl+Na<sub>2</sub>SO<sub>4</sub> (1:1) : control (●), 100 mM (○), 300 mM (▼), 500 mM (△), 700 mM (■), 1000 mM (□)



**Figure 2.** Level of osmoprotectants in *Anabaena* sp. BHUAR002 at different time interval with 200 mM NaCl (a) and NaCl+Na<sub>2</sub>CO<sub>3</sub> (1:1) (b).

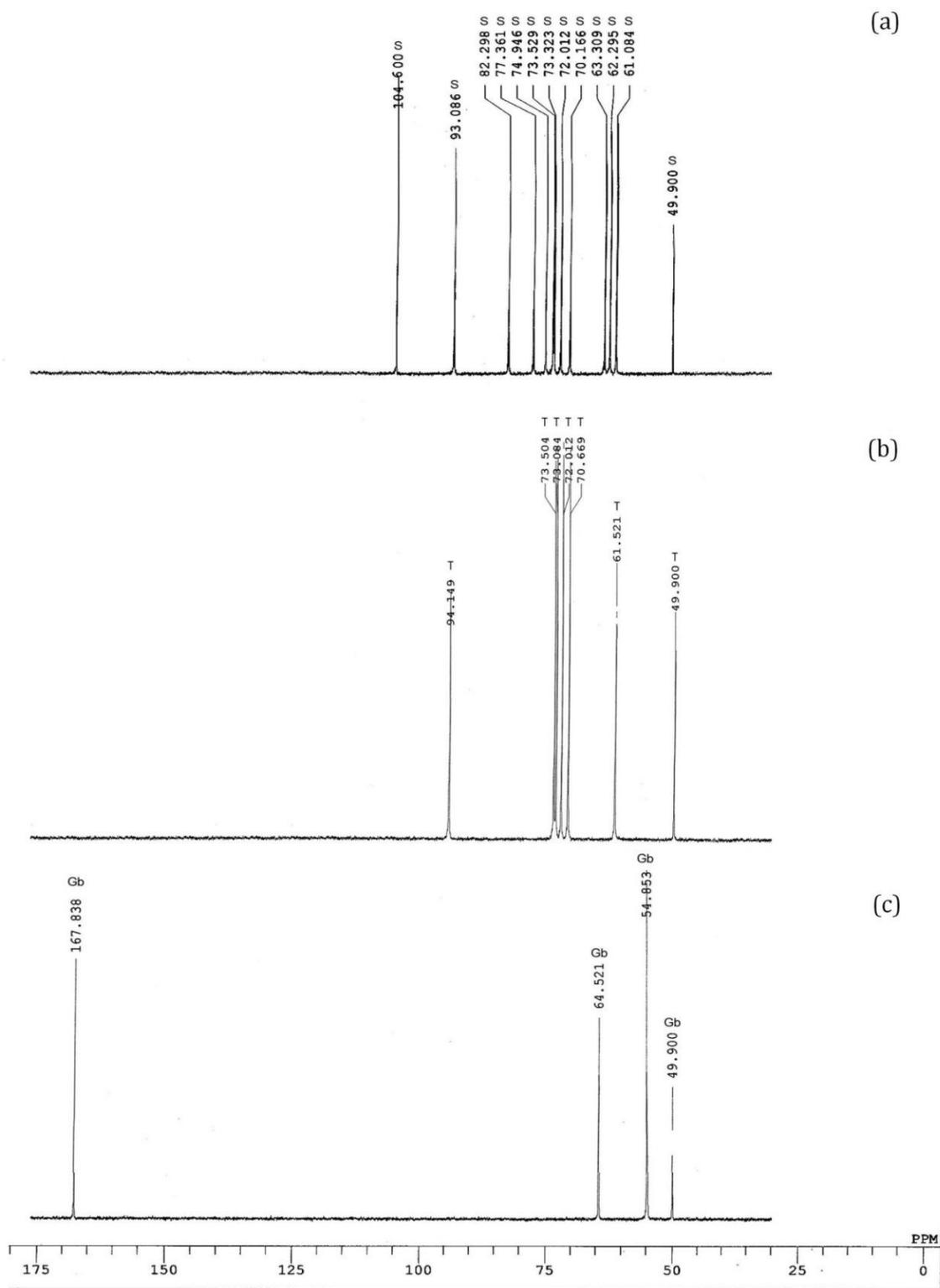
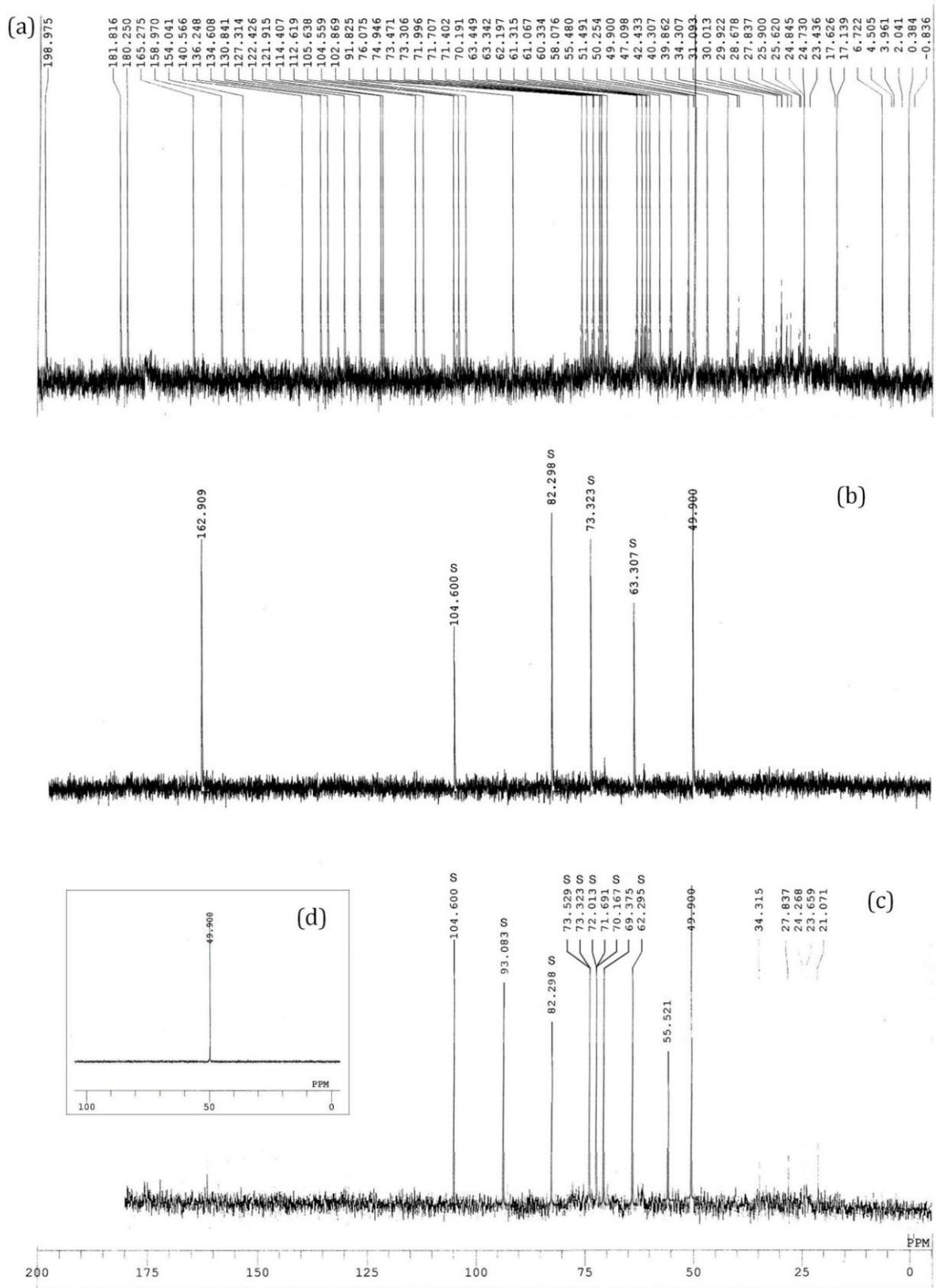


Figure 3.0.  $\delta$ ppm shift ( $^{13}\text{C}$ -NMR Scale) of the standard of Sucrose (a), Trehalose (b) and Glycine betaine (c).



**Figure 4.0.** a, b, c, d are showing  $^{13}\text{C}$ -NMR results ( $\delta$ ppm shift) of  $\text{NaCl}+\text{Na}_2\text{SO}_4$  (1:1) stressed,  $\text{NaCl}+\text{Na}_2\text{CO}_3$  (1:1) stressed,  $\text{NaCl}$  stressed, and control samples respectively.

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## THE FIELD SCREENING OF THE SOMATIC EMBRYOGENESIS CULTURES DERIVED COCOA CLONE TREES FOR THE RESISTANCE TO VASCULAR STREAK DIEBACK (VSD) DISEASE

Entuni, G.,\* Edward, R., Nori, H. and Ahmad Kamil, M.J.

<sup>1</sup>Plant Science and Environmental Ecology, Faculty of Resource Science and Technology, Universiti Malaysia Sarawak, 94300 Kota Samarahan, Sarawak, Malaysia

<sup>2</sup>Malaysian Cocoa Board, Cocoa Research and Development Centre, Lot 248, Blok 14, Biotechnology Park, 94300, Kota Samarahan, Sarawak  
Email: [gib5181@gmail.com](mailto:gib5181@gmail.com)

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**Abstract:** Vascular streak dieback (VSD) caused by the fungus *Oncobasidiumtheobromae* is a devastating pathogen of cocoa (*Theobroma cacao* L.). This disease effects both young seedlings and mature trees. Plant tissue culture technique viz. somatic embryogenesis has a potential to overcome this problem by the development of VSD disease resistant cocoa planting materials. To ensure the effectiveness of this technique, the field screening of resistant of the regenerated cocoa clone trees to VSD was evaluated. The method used was field observation based on visual scoring of VSD infection under normal planting conditions. Thirty cocoa plants derived from immature zygotic embryo and 30 cocoa plants derived from staminode explants of Trinitario varieties were planted in field condition. Pruning to remove the infected branches was carried out to determine the relationship between characteristics of sprouting ability and VSD scoring of the severity for each regenerated cocoa clone trees. At one year of planting, it was found that immature zygotic embryo cultures derived cocoa trees were resistant than staminode cultures derived cocoa trees to VSD disease. The MCBC1 cocoa clone trees either derived from immature zygotic embryo culture or staminode cultures showed the optimum characteristics of sprouting ability than other type of cocoa clone trees.

**Keywords:** *Theobroma cacao*, Tissue culture, Somatic embryogenesis, Field experiment, Vascular streak dieback

### INTRODUCTION

*Theobroma cacao* L. or simply known as cocoa tree is one of the most important cash crop trees grown in the humid tropics. In the last decade, its consumption has increased as cocoa is cultivated for its fruit in which the seeds are used for the production of chocolates and confectionaries. Conventionally, cocoa can be propagated through seed, rooted cuttings of buddings from plagiotropic or orthotropic shoots, marcotting and grafting. However, cocoa propagation through seed generates high genetic variability due to its natural propagation system of allogamous. A high degree of yield variation often found in cocoa plantation of using seeds as the method of propagation (Maximovaet al., 2002). To minimize the negative effect through seed propagation, regeneration of cocoa through rooted cutting, marcotting and grafting are frequently used. Though, rooted cutting, results in a sprawling, bush-like architecture lacking the normal dimorphic growth habit. The regenerated trees required extensive pruning to achieve a more convenient shape for harvesting and other farm operations. These methods of propagation has been described as inefficient and costly (Figueira and Janick, 1995).

Recently, plant regeneration through somatic embryogenesis offers an alternative approach to overcome these conventional propagation methods of cocoa. In cocoa, somatic embryogenesis is the most

frequently adopted *in vitro* technique, which has been used not only for plant propagation but also for genetic engineering (Loyola and Vasquez, 2006), virus elimination (Quainoo and Dwomon, 2012; Edward and Wetten, 2016) and germplasm preservation (Maximovaet al. 2002). The diseases have been eliminated from various crops such as oil palm (*Elaeisguineensis* L.), banana (*Musa* L. sp.), apple (*Malus* Mill. sp.), Barley (*HordeumVulgare* L.), cherry (*Prunusaviumxpseudocerasus* L.) and pear (*Pyruscommunis* L.) (Gorbarenko&Zhuk, 1972; Hartman, 1974; Grout, 1999) throughplant tissue culture technique of somatic embryogenesis.

In cocoa, VSD is a serious disease that destroyed most cocoa plantations worldwide. This disease was associated with the windborne basidiomycetesfungus known as *O. theobromae*. The infestation of this basidiomycetes pathogen begins by infecting the new leaves of young cocoa trees. The fungus then moves to the xylem, resulting in vascular browning among the veins of the lamina. After that, the disease spreads to the midrib and petiole of the leaves and finally reaching the branch. The symptoms of VSD disease include the leaf chlorosis with green spots, necrotic leaf scars from abscised leaves with 3 dots or as dark streak when stems are split open, rough bark as a result of swollen lenticels immediately below the petiole of the affected leaves, “broomstick” symptoms as a result of proliferation and subsequent death of axillary buds following

\*Corresponding Author

leaves abscission (Keane, 1981; Guest and Keane, 2007; Samuels *et al.* 2012).

As *C. theobromae* is a vascular pathogen, control by fungicides is hampered with difficulty, and although effective systemic fungicides such as Triazole, Flutriafol, Hexaconazole, Propiconazole and Triadimenol have been used, they are generally too costly for smallholder farmers (Minimolet *et al.* 2015). To date, cultural practices to control VSD are by using clean nursery stocks, canopy pruning of disease infected trees and through shade and canopy management to increase aeration and sunlight exposure to the infection sites. Among these cultural practices, canopy pruning to control VSD infection is much preferred. It was reported that canopy pruning to remove all VSD tissues of  $\pm 10$  cm beyond the streaking infections of VSD carried out every two to three weeks able to control the disease (Susilo and Sari, 2014). The canopy pruning of the infected cocoa branches about  $\pm 10$  cm below the discolored xylems inhibits further spreading of the infection and decreases inoculum level by removing potential sites of sporulation (Guest and Keane, 2007). It was studied that when VSD infected cocoa trees were left unpruned, the disease incidence rise from about 30 to 90% within 10 months period (Guest and Keane, 2007). Minimolet *et al.* 2015 reported that the use of systemic fungicides are ineffective in controlling VSD diseases and the only viable solution is to regenerate resistant cocoa planting materials.

Another option for effective long term solution for VSD control is through the introduction of the resistant cocoa planting materials of high quality. This can be potentially achieved through resistance plant breeding programme through application of somatic embryogenesis technique. The use of the disease resistant cocoa clone could decrease the usage of chemicals from pesticides for disease

control thus beneficial to the environment, reduce the carbon emission if using machineries for the application of pesticides as well as useful for soil conservation. Besides, for the smallholder farmers, the cost of pesticides application can be minimized. Nevertheless, as not all plants regenerated *in vitro* are guaranteed free of diseases, therefore scoring and screening of the regenerated somatic embryogenesis cultures derived cocoa plants for the occurrence of disease such as VSD during early stage of field planting is crucial. In this study, the relationship between characteristics of sprouting ability and VSD scoring of the severity for each regenerated cocoa clone trees were evaluated. The characteristics of sprouting ability was evaluated by recording the pruned somatic embryogenesis cultures derived cocoa trees for the variables of the number of the regrowth shoot, shoot length, number of new shoots per pruned branch, shoot diameter and number of leaves per shoot.

## MATERIAL AND METHOD

### Experimental site and plant materials

This study was conducted from April 2016 to August 2017 in MCB Kota Samarahan, Sarawak, Malaysia involved 30 Trinitario variety cocoa clone trees from immature zygotic embryo cultures and also 30 Trinitario variety cocoa clone trees derived from staminode cultures. The soil in the area of the study was classified as Histosols and experiences an equatorial climate which is characterized by hot and humid conditions with a drier period during the middle of the year and high rainfalls at the end of the year (Mohamad Tarmiziet *al.* 2014). The area has an annual average temperature of 27.5°C, relative humidity of 88.0% and a mean monthly rainfall ranging between 19.1 to 310 mm.

**Table 1.** Total number of staminode cultures trees and immature zygotic embryo cultures trees assessed in the field for the scoring on VSD disease severity.

Type of somatic embryogenesis derived cocoa trees	Type of cocoa clone trees	Total
Staminode cultures derived cocoa trees	BR25	6
	PBC230	6
	KKM4	6
	KKM22	6
	MCBC1	6
Immature zygotic embryo derived cocoa trees	BR25	6
	PBC230	6
	KKM4	6
	KKM22	6
	MCBC1	6

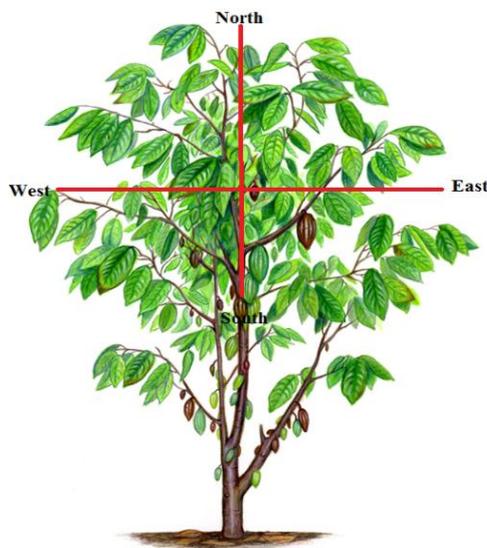
### Scale of assessment for VSD infection after one year of field planting

The data on the scoring for the severity of each regenerated somatic embryogenesis cultures derived cocoa trees was collected based on method described by Ahmad Kamilet *al.* (2016). The scale used for the

scoring on VSD disease severity was ranging from 0 to 6 (Table 2) based on the cocoa VSD progressive infection on the cocoa branches in April 2017 after one year of field planting. These severity scale were used to classify the cocoa clone into four groups of resistance comprised of susceptible (severity score

between 4.0 to 6.0), moderately susceptible (severity score between 3.0 to 3.9), moderately resistant (severity score between 2.0 to 2.9) and resistant (severity score between 1.0 to 1.9) (Susilo and Anita

Sari, 2014; Ahmad Kamilet *et al.* 2016). Four parts of plant canopies with the infected branches and leaves (Figure 1) were evaluated for the mean of the severity of VSD infections.



**Figure 1.** Diagram of the four part of plant canopies for the VSD disease assessment (Ahmad Kamilet *et al.* 2016).

**Table 2.** Score of severity damage based on the primary symptoms due to VSD infection of somatic embryogenesis cultures derived cocoa trees for field screening (Adapted from Ahmad Kamilet *et al.* 2016).

Severity score	Primary symptoms	Other associated symptoms
0	Uninfected trees	Smooth bark
1	<25% of cocoa plant branches and leaves were infected by VSD but no effect on plant vigor	Smooth bark with or without swollen lenticels
2	>25-50% of cocoa plant branches and leaves were infected by VSD that tend to result in vigor declining such as leaves turn chlorosis	Moderately smooth bark with slightly swollen lenticels
3	>50-75% cocoa plant branches and leaves were infected by VSD which slightly affect vigor in which most of the infected leaves turn chlorosis and necrotic but still remain attached in the branches	Moderately rough bark with slightly swollen lenticels
4	>75% cocoa plant branches and leaves were infected by VSD which tend to seriously affect vigor where the infected leaves begin to abscise	Moderately to very rough bark with slightly swollen lenticels
5	Most of the cocoa plant branches and leaves were infected by VSD which significantly affect vigor as most of the infected leaves have abscised	Very rough bark with the presence or absence of fruiting bodies
6	The infected plants were seriously damaged by VSD, some of those infected plants were died. Dieback occurred and infected plant part was died	Very rough bark without proliferation of auxiliary shoots and with presence VSD of fruiting bodies

### Pruning procedures

At the same time, pruning was carried out to remove the four parts of the infected branches of around  $\pm 10$  cm beyond the streaking infections based on modified method from Susilo and Sari (2014). The pruning was done for the three moderately resistance cocoa clone trees both regenerated through immature zygotic embryo and staminode cultures such as KKM4, BR25 and KKM22 (Lee *et al.* 1993; Malaysian Cocoa Board, 2005) as well as for the susceptible cocoa clone, the PBC230 (Lee *et al.* 1993; Malaysian Cocoa Board, 2005) cocoa clone trees that have been infected by VSD disease. On the other hand, MCBC1 as the resistance cocoa clone

trees (Lee *et al.* 1993; Malaysian Cocoa Board, 2005) was also pruned and used as a control tree. A total of 12 parts (North, East, West and South parts) of each cocoa clone trees types infected branches were removed and pruned in this study. The pruning was done during dry season to minimize the incidence of the disease which correlated with high rainfall rate as found in the wetter areas, the spreading of the VSD disease will be higher than those in the dry area. The systemic fungicide such as Bayfidan also applied after pruning.

### Pruning for the characteristics of sprouting ability

Assessment on the characteristics of sprouting ability for the variables of the number of the regrowth shoot, the length of the longest shoot, number of new shoots per pruned branch, the diameter of the longest shoot and number of leaves per shoot of each somatic embryogenesis cultures derived cocoa clone trees were evaluated after three months of pruning. The standard agronomy practices were carried out during the period of the evaluation with the application of the complex fertilizer with NPK contents of 12:12:17+TE. The application of fertilizer of around 20 to 30 gram per tree was done after pruning.

#### Data collection and analysis

The data on the characteristics of sprouting ability for the variables of the number of the regrowth shoot, shoot length, number of new shoots per pruned branch, shoot diameter and number of leaves per shoot of each somatic embryogenesis cultures derived cocoa clone trees were assessed after three months of pruning. For these data, only the normal shoot without VSD infections were further evaluated. Mean of data collected for the characteristics of the sprouting ability for each of the cocoa clone trees were analyzed using analysis of variance (ANOVA) test and if there is a significant difference detected then it is further analyzed with Fisher Protected LSD test at  $p < 0.05$  significance level using SPSS Software Version 22.

## RESULT AND DISCUSSION

### Scale of assessment for VSD infection after one year of field planting

Four parts of cocoa plant canopies with the infected branches and leaves (Figure 2) were evaluated in April 2017 after one year of field planting for the incidence of VSD infections. From data collected (Table 3), MCBC1 cocoa clone trees showed the lowest mean of severity scale for assessment when compare to others type of cocoa clone trees. Though, the MCBC1 cocoa clone trees derived from immature zygotic embryo were found more resistant

than the MCBC1 cocoa clone trees derived from staminode cultures (1.36) as the mean severity scale for VSD infection was the lowest (1.11). MCBC1 cocoa clone was one of the most VSD resistant cocoa clone and recommended for the used as planting materials with the high yielding capability (MCB, 2015). An overall for all of the cocoa clones, the mean severity scale of VSD infection for immature zygotic embryo derived cocoa trees were also lowest as compare to the mean severity scale of the staminode cultures derived cocoa clone trees. Susilo and Anita Sari (2014) reported that cocoa clone have varying degree of tolerance to VSD disease depending on their genetic background.

Among cocoa clone trees evaluated, PBC230 cocoa clone trees from both somatic embryogenesis cultures showed the highest mean severity scale of VSD infection after one year of field planting. The primary symptoms of the VSD infection in PBC230 cocoa clone trees during the field inspection including leaf turn yellow and chlorosis (Figure 3a), rough bark (Figure 3b) and swollen leaf lenticel (Figure 3c). Under International Cocoa Gerplasm Database (2017), the PBC230 cocoa clone is categorized as VSD moderately resistant nevertheless frequently planted for its promising high yielding and quality of seeds. For the moderately resistant cocoa clone trees, cultural practice such as pruning was carried out in order to open the canopy to increase air circulation and reduce the humidity. Reducing the humidity is crucial to avoid spore formation, sporulation and infection of *O. theobromae* (Voset *al.* 2003). This study revealed that the staminode cultures derived cocoa trees presented the highest mean severity scale of VSD infection than immature zygotic embryo cultures trees. Hence, from this study it was proved that cocoa clone trees regenerated from immature zygotic embryos cultures were better than cocoa clone trees regenerated from staminode cultures in term of field performance against VSD infection after one year of field planting.

**Table 3.** Mean VSD disease severity scale of the immature zygotic embryo cultures derived cocoa trees and staminode cultures derived cocoa trees after one year of field planting.

Type of somatic embryogenesis derived cocoa trees	Type of cocoa clone trees	Mean severity scale for VSD infection
Staminode cultures derived cocoa trees	BR25	1.88
	PBC230	3.88
	KKM4	2.04
	KKM22	1.67
	MCBC1	1.36
Immature zygotic embryo derived cocoa trees	BR25	1.60
	PBC230	3.22
	KKM4	2.00
	KKM22	1.44
	MCBC1	1.11



**Figure 2.** The white circles showed the four parts of cocoa plant canopies with the infected branches and leaves assessed for the incidence of VSD infections in which (a) PBC230 cocoa clone tree regenerated from staminode culture; (b) PBC230 cocoa clone tree regenerated from immature zygotic embryo culture.



**Figure 3.** The primary symptoms of the VSD infection in PBC230 cocoa clone trees during field inspection after one year of field planting in April 2017 where (a) leaf turn yellow and chlorosis; (b) rough bark; (c) swollen leaf lenticel.

### **Pruning for the characteristics of sprouting ability**

#### **Staminode cultures derived cocoa trees**

The data on the characteristics of sprouting ability (Table 4) for staminode cultures derived cocoa trees was evaluated after three months of pruning in July 2017 after the removal of the VSD infected branches of around  $\pm 10$  cm beyond the streaking infections (Figure 4a). The healthy shoots and healthy branches without any VSD infections on the cut surface were used for the evaluation (Figure 4b). BR25 and MCBC1 cocoa clone trees showed the highest percentage of the sprouting branches (100%). The PBC230 cocoa clone trees presented the lowest percentage of the sprouting branches of around 83.3%. It was found that there was a significant different between the mean number of the emerge

leaves for MCBC1 cocoa clone trees with the other type of staminode derived cocoa clone trees. The MCBC1 cocoa clone trees recorded the highest mean number of the emerged leaves of  $5.92 \pm 0.29$  whereas KKM22 recorded the lowest mean number of the emerge leaves of  $3.75 \pm 0.18$ . From this study, it was proved that the number of sprouting branches and the number emerge leaves were correlated with the mean severity scale to VSD infection. The mean number of emerge leaves were higher for the resistant cocoa clone tree with the lowest mean scoring of VSD such as the MCBC1 cocoa clone. The similar finding was also obtained by Susilo and Sari (2014) in Indonesia in which the VSD resistant cocoa clone trees have the optimum characteristics of sprouting ability than the moderately resistant cocoa clone trees after pruning.

In addition, for the mean number of shoot, the MCBC1 cocoa clone trees also recorded the highest mean number of shoot as compare to other type of cocoa clone trees in which the mean number was around  $3.25 \pm 0.13$ . The KKM22 cocoa clone trees showed the lowest mean length of the longest shoot with the mean length of  $6.68 \pm 0.22$  whereas the MCBC1 showed the highest mean length of the longest shoot of around  $15.38 \pm 0.33$ . Besides, there was also a significant different among mean diameter of the longest shoot for each of the cocoa clone trees derived from this staminode cultures. The mean diameter of the longest shoot was ranged from  $1.20 \pm 0.55$  to  $2.31 \pm 0.92$ . The KKM22 cocoa clone

trees mean diameter of the longest shoot was significantly different with other type of cocoa clone trees and this cocoa clone tree recorded the lowest mean diameter of around  $1.20 \pm 0.55$ . In contrast, this mean diameter of the longest shoot was double in MCBC1 cocoa clone trees in which the mean diameter recorded was around  $2.31 \pm 0.92$ . Based on this finding, it was proved that MCBC1 cocoa clone trees showed the superior characteristics of sprouting ability among other staminode derived cocoa clone trees based on the evaluation of the characteristics after three months of pruning to remove VSD infected branches.

**Table 4.** Pruning for the characteristics of sprouting ability among staminode derived cocoa clone trees after three months of pruning.

Type of somatic embryogenesis derived cocoa trees	Type of cocoa clone trees	Percentage of the sprouted branches (%)	Mean number of emerge leaves $\pm$ SE	Mean number of shoot $\pm$ SE	Mean length of the longest shoot $\pm$ SE (mm)	Mean diameter of the longest shoot $\pm$ SE (mm)
Staminode cultures derived cocoa trees	BR25	100	$4.75 \pm 0.22^a$	$2.42 \pm 0.14^a$	$12.17 \pm 0.43^a$	$2.26 \pm 0.12^a$
	PBC230	83.3	$4.17 \pm 0.32^a$	$2.42 \pm 0.14^a$	$11.10 \pm 0.27^a$	$2.01 \pm 0.98^a$
	KKM4	91.6	$4.67 \pm 0.26^a$	$2.17 \pm 0.11^{ab}$	$9.38 \pm 0.43^b$	$2.10 \pm 0.11^a$
	KKM22	91.6	$3.75 \pm 0.18^a$	$1.67 \pm 0.19^b$	$6.68 \pm 0.22^c$	$1.20 \pm 0.55^b$
	MCBC1	100	$5.92 \pm 0.29^b$	$3.25 \pm 0.13^c$	$15.38 \pm 0.33^d$	$2.31 \pm 0.92^a$

Mean characteristics of sprouting ability among staminode derived cocoa clone trees after three months of pruning. Means with the same letter are not significantly different at  $p \geq 0.05$  based on LSD test.



**Figure 4.** The PBC230 cocoa clone tree infected by VSD after one year of field planting. (a) The VSD infected branches of around  $\pm 10$  cm beyond the streaking infections in April 2017; (b) The PBC230 cocoa clone tree new shoot sprouted after one month of pruning in May 2017.

#### Immature zygotic embryo cultures derived cocoa trees

All of the pruned branches of cocoa clone trees of the BR25, KKM22 and MCBC1 were found sprouted after three months of pruning for the removal of VSD infected branches. On the other hand, the PBC230 and KKM4 cocoa clone trees recorded the lowest percentage of the sprouted branches of around 91.6% respectively. The maximum mean number of emerge leaves was discovered in MCBC1 cocoa clone trees with around  $6.83 \pm 0.72$  whereas the minimum mean number of emerge leaves was found in KKM22 cocoa clone trees with mean number of emerge leaves of around  $3.92 \pm 0.90$ . As obtained from LSD

test, there was a significant different among cocoa clone trees in term of the mean number of the emerge leaves. The KKM22 showed the lowest mean number of shoot of around  $1.75 \pm 0.22$  while MCBC1 showed the highest mean number of shoot of around  $3.50 \pm 0.15$ . The other cocoa clone trees recorded mean number of shoot of around  $2.83 \pm 0.11$  for BR25 cocoa clone trees,  $2.58 \pm 0.19$  for KKM4 cocoa clone trees and  $2.25 \pm 0.13$  for PBC230 cocoa clone trees. The mean length of the longest shoot discovered were ranged around  $6.73 \pm 0.10$  to  $16.47 \pm 0.20$  in which the maximum mean of the longest shoot was found in MCBC1 cocoa clone trees whereas the minimum mean of the longest shoot was recorded in

KKM22. Bases on the LSD test, there was a significant different in term of the mean length of the longest shoot among these immature zygotic embryo derived cocoa clone trees. The mean diameter of the longest shoot was also studied in which it was found that the MCBC1 cocoa clone trees presented the highest mean diameter of the longest shoot of around

3.26±0.10. This followed by BR25 cocoa clone trees with mean diameter of the longest shoot of 2.82±0.58, PBC230 of around 2.91±0.07 mean diameter of the longest shoot and KKM4 with 2.71±0.07 mean diameter of the longest shoot. The KKM22 cocoa clone trees presented the lowest mean diameter of the longest shoot of around 1.62±0.05.

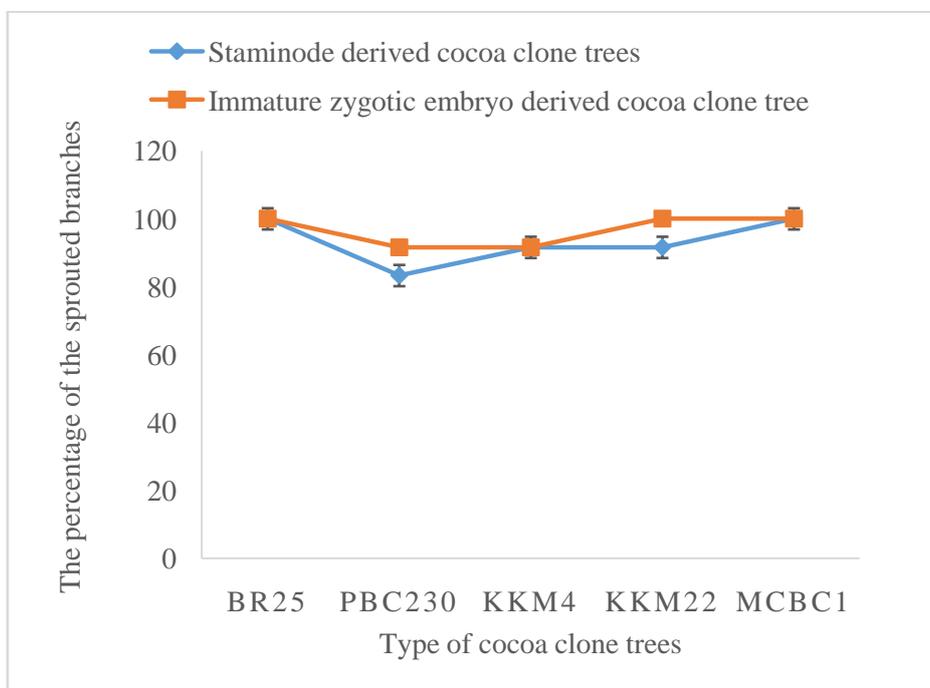
**Table 5.** Pruning for the characteristics of sprouting ability among immature zygotic embryo derived cocoa clone trees after 3 months of pruning.

Type of somatic embryogenesis derived trees	Type of cocoa clone trees	Percentage of the sprouted branches (%)	Mean number of emerge leaves ± SE	Mean number of shoot ± SE	Mean length of the longest shoot ± SE (mm)	Mean diameter of the longest shoot ± SE (mm)
Immature zygotic embryo derived cocoa trees	BR25	100	4.41±0.67 <sup>ab</sup>	2.83±0.11 <sup>a</sup>	13.20±0.31 <sup>a</sup>	2.82±0.58 <sup>a</sup>
	PBC230	91.6	4.50±1.17 <sup>a</sup>	2.25±0.13 <sup>ab</sup>	11.46±0.22 <sup>b</sup>	2.91±0.07 <sup>a</sup>
	KKM4	91.6	5.08±0.67 <sup>a</sup>	2.58±0.19 <sup>a</sup>	10.18±0.44 <sup>c</sup>	2.71±0.07 <sup>a</sup>
	KKM22	100	3.92±0.90 <sup>b</sup>	1.75±0.22 <sup>b</sup>	6.73±0.10 <sup>d</sup>	1.62±0.05 <sup>b</sup>
	MCBC1	100	6.83±0.72 <sup>c</sup>	3.50±0.15 <sup>c</sup>	16.47±0.20 <sup>e</sup>	3.26±0.10 <sup>c</sup>

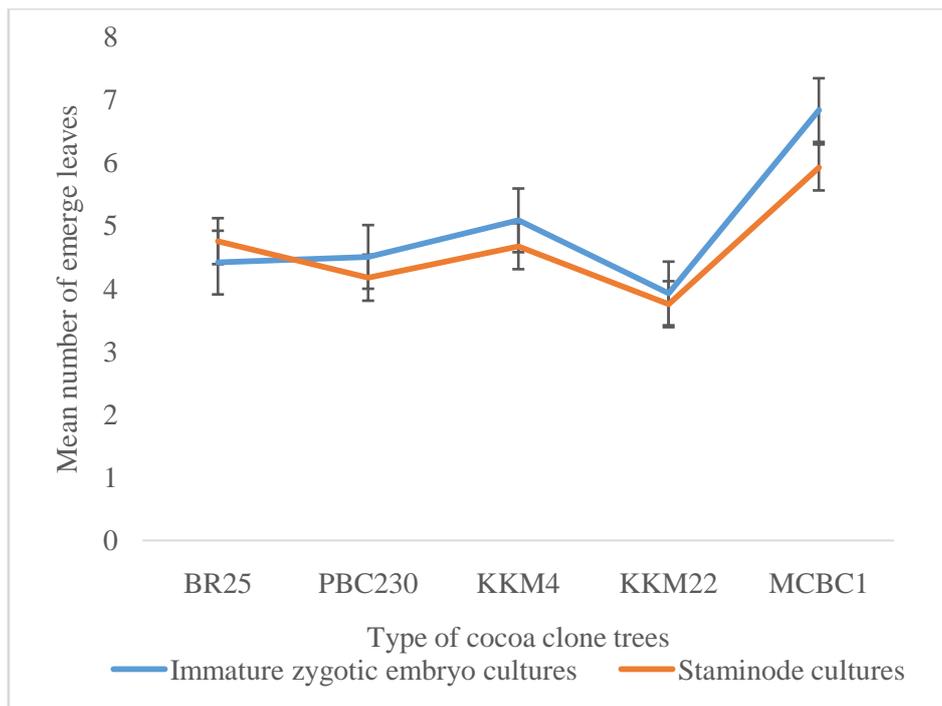
Mean characteristics of sprouting ability among immature zygotic embryo derived cocoa clone trees after three months of pruning. Means with the same letter are not significantly different at  $p \geq 0.05$  based on LSD test.

Hence, in this study the cocoa clone trees derived from immature zygotic embryos cultures showed the optimal characteristics of sprouting ability such as percentage of the sprouted branches (Figure 5), mean number of emerge leaves (Figure 6), mean number of

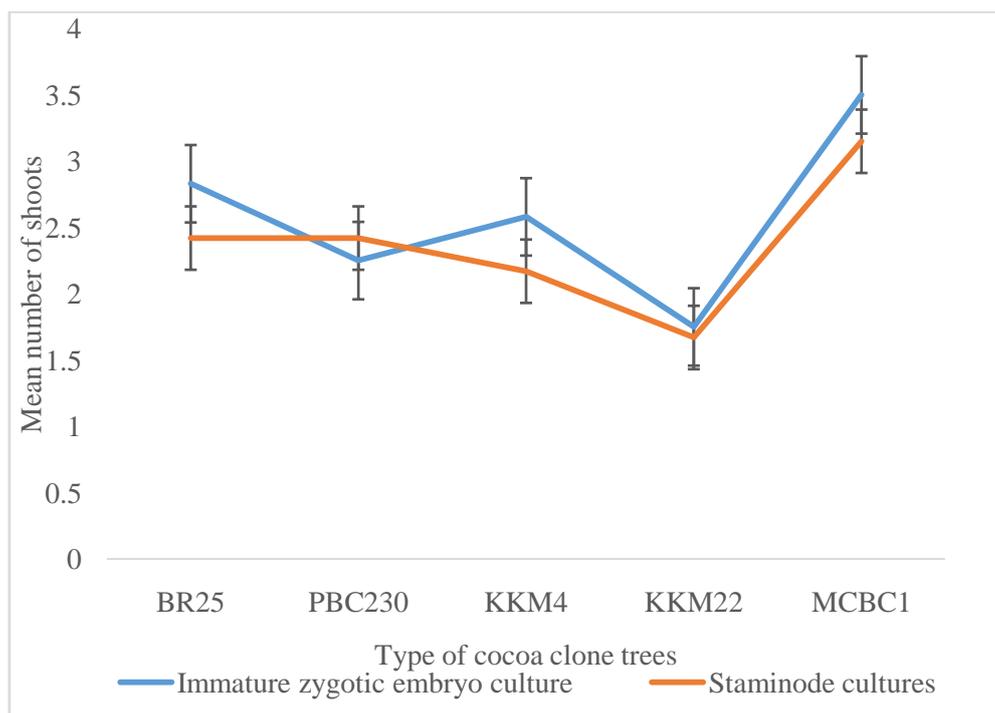
shoot (Figure 7), mean length of the longest shoot (Figure 8) and mean number of longest shoot (Figure 9) as compare to cocoa clone trees derived fromstaminodecultures after three months of pruning.



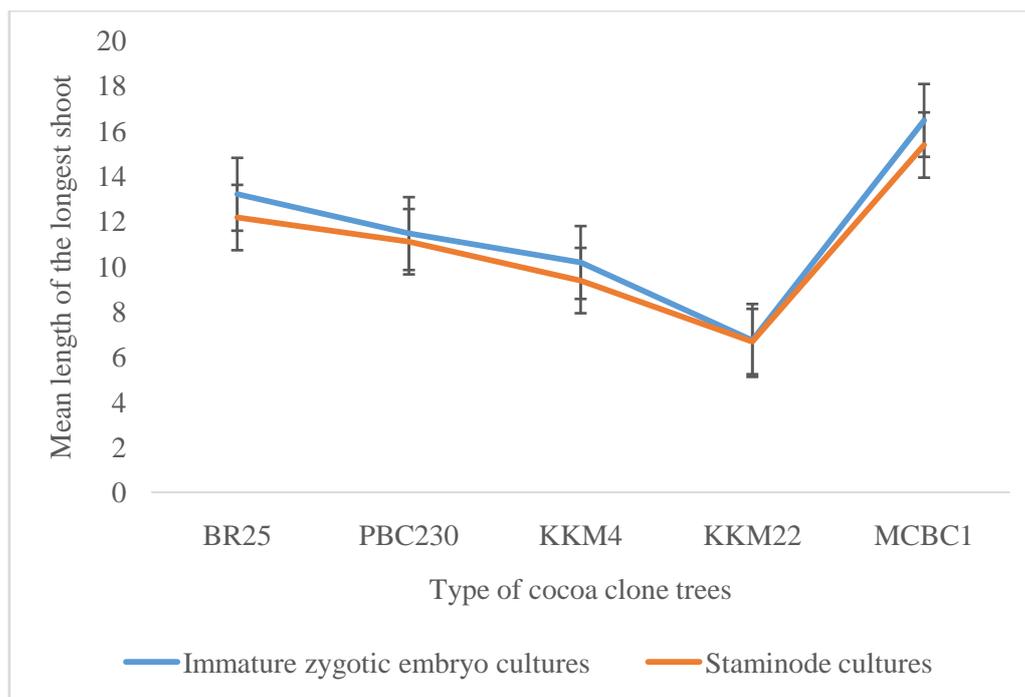
**Figure 5.** The percentage of the sprouted branches among cocoa clone trees derived from both staminode and immature zygotic embryo cultures.



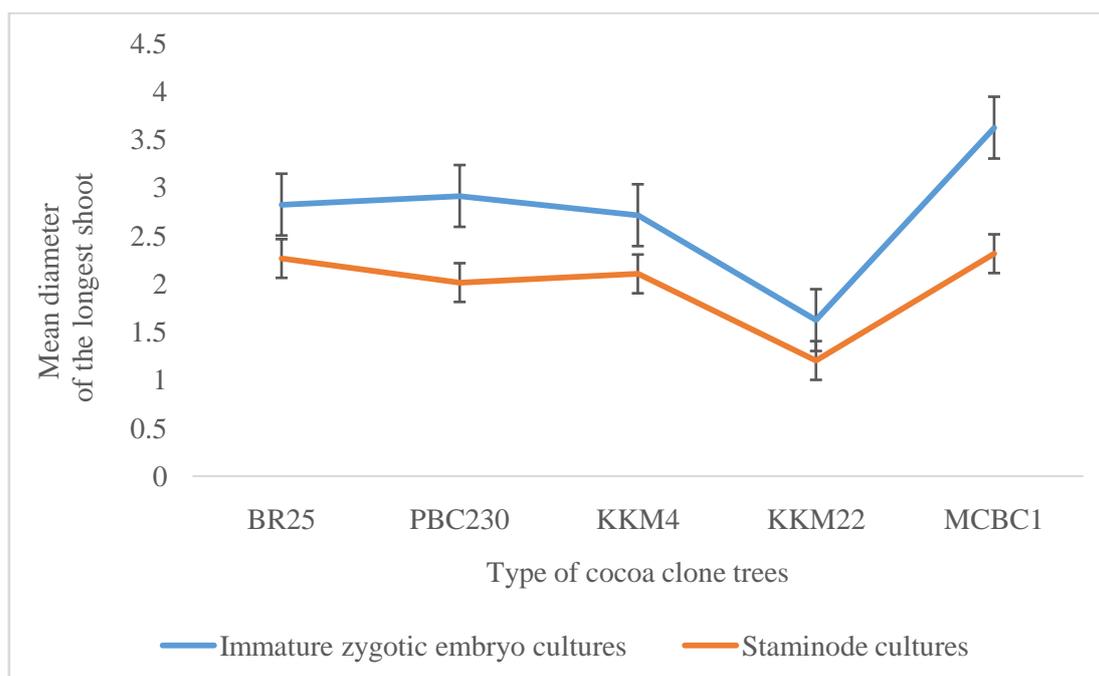
**Figure 6.** Mean number of emerge leaves of staminode and immature zygotic embryo cultures derived cocoa trees after three months of pruning.



**Figure 7.** Mean number of shoots of staminode and immature zygotic embryo cultures derived cocoa trees after three months of pruning.



**Figure 8.** Mean length of the longest shoots of staminode and immature zygotic embryo cultures derived cocoa trees after three months of pruning.



**Figure 9.** Mean diameter of the longest shoots of staminode and immature zygotic embryo cultures derived cocoa trees after three months of pruning.

**CONCLUSION**

After one year of field planting, the immature zygotic embryo cultures derived cocoa clone trees were proved to be resistant to VSD disease than the staminode cultures derived cocoa clone trees. Among five of the cocoa clone trees evaluated, MCBC1 released for commercial planting as VSD resistant cocoa clone have the lowest mean severity scale to

VSD infection either derived from staminode cultures or immature zygotic embryo cultures. From this finding, the characteristics of the sprouting ability of each cocoa clone trees were depending on the genotype in which the VSD resistance cocoa clone tree such as MCBC1 was more superior to moderately resistant cocoa clone trees such as PBC230 and KKM4 in term of sprouting characteristics after three months of pruning. The

result revealed that pruning play an important roles on the management of VSD disease in cocoa.

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## PRESENT STATUS AND FUTURE PROSPECTS OF FISHERIES DEVELOPMENT IN BIHAR WITH SPECIAL REFERENCE TO SOME SELECTED OX-BOW LAKES OF MUZAFFARPUR DISTRICT

Satyendra Kumar\*

*Scientist Fishery, Krishi Vigyan Kendra, East Kameng Arunachal Pradesh*

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**Abstract:** Total fish Farmers Development agency (FFDA) working in the state is 33. Water area development through this is around 26,000 ha and average annual productivity of ponds /tanks under this 2175 kg/ha/yr. The main components of work under this centrally sponsored scheme are construction of new ponds, renovation of old derelict ponds through bank finance, training to farmers, supply of essential inputs and extension support. Total no. of ox-bow lakes in the state is 63 which become well. Besides these are many ox-bow lakes which have either become extinct due to certain bio-geological phenomenon or in the process of extinction. There are some lakes with process of formation has been halted due to the raising of earthen embankment, a measures of flood control, and as such these lakes remain in half formed state but still have opened connection with the parent river, resulting into complete inundation during monsoon months. However, they served as good resources for capture fisheries, being a collection sink of riverine stock during the flood

**Keywords:** Future prospects of fisheries, Development in Bihar and Muzaffarpur District

### INTRODUCTION

In the developing countries, fish constitutes one of the single largest cheap source of animal protein. Fish protein is a relatively high digestibility and biological value for human beings, as it contains all the essential amino acids in adequate amount and balanced proportion. There is no doubt that fish could make a more significant to nutrition, particularly among undernourished. Indian fisheries have made great strides during the past five decades. As a result, India now produces over 6.1 million tones of fish and shellfish from capture fisheries and aquaculture, thereby contributing immensely to the food basket of the country. During this period, fish production has registered over eight- fold increase, from 0.75 million tonnes in 1950. Further, the share of inland fisheries sector in the total fish production, which was 29% in 1950-51, has gone up by 50% at present. India being the fourth largest global producer of fish, is playing an important role in world fisheries scenario. Further, with a production of over 2.2 metric million tonnes from aquaculture as in 2000, the country occupies second position in the world in inland fish production, only next to China. The aquaculture sector has shown overwhelming growth of 46.8% during the last two decades i.e., from 0.37 million tonne in 2002. Further, fresh water continues to have a major share out of total aquaculture production with contribution of over 95% in terms of quantity ( Ayyapan and Jena, 2003).

As far as inland fisheries resources is concerned, India is blessed with huge freshwater aquaculture resources consisting of 2.36 million ha of ponds and tanks, 1.07 million km of canals, 3.15 million ha of reservoirs and 0.72 million ha of upland lakes that could be put to different fish culture practices or even

culture based capture fisheries in case of large water bodies. Available statistics show that only about 0.8-0.9 million ha of available water area under ponds and tanks have been put to use for aquaculture across the country at present. In spite of availability of huge open inland water resources in the country, the contribution from inland capture fisheries is of the order of 0.7 million tonnes out of total inland production of a little over 2.2 million tonnes from freshwater aquaculture indicates that the sector in overall terms, possesses a potential of producing over 4.5 million tonnes annually, through adoption of appropriate technologies, effective transfer of scientific knowledge and provision of required critical inputs quantity (Ayyappan and Jena, 2003). After creation of Jharkhand state (15<sup>th</sup> November 2000), Bihar has lost a sizeable extent of water areas in the form of reservoir. At present only 30% of large reservoir in the residual Bihar. As far as fisheries resources in the state of Bihar is concerned, they mainly comprise ponds, tanks, small reservoirs, rivers and water logged areas like ox-bow lakes and chauras. Around 65,000 ha of water areas are covered by ponds and tanks and nearly 35,000 ha of water areas consists of ox-bow lakes and chauras. At present annual production of fish in the state is 2.2-2.5 lakh tonne, while average annual production of fish seed is 350 million numbers against the requirement of 600 million fry per year. There are 18 hatcheries in the state; one in government sector, 03 in corporate sector and 14 in private sector. The functional FFDA's in the state is 33.

### MATERIAL AND METHOD

The present investigation of water resources and their fish production was conducted in the Muzaffarpur district of Bihar. This district has 16 numbers of

\*Corresponding Author

blocks but the survey was done in Mushari, Motipur and Kanti block which are prominent in fish production. The town / village selected were Brahamputra and Manika under Mushari block and Haruna and Kanti Village Under Motipur Block respectively. Besides discussion with various officials in Fisheries Directorate, Bihar were also done to collect more information. The primary survey was conducted in Mushari, Motipur and Kanti block of Muzaffarpur district. This district is selected for the survey because it is bestowed with series of ox-bow lakes and among them some lakes like Manika, Brahampura, Motipur and Kanti are highly significant from fisheries point of view. These ox-bow lakes have define bearing on socio-economic conditions of the area, being one of the major sources of livelihood for thousands of fisherman living in their vicinity. This district has second highest water area of ox-bow lakes after east and West Champaran district of Bihar. All above four selected ox-bow lakes are located in different corner of Muzaffarpur district. Manika lake is 13 km east of Muzaffarpur town, while Kanti and Motipur lake located in the 16 km and 36 km respectively in the west of Muzaffarpur town on Muzaffarpur –Raxaul highway. The Brahampura lake is located in the city itself. In Mushari block, selected villages were Brahampura and Manika lake while in Motipur and Kanti block, the selected villages were Harayana and Kanti respectively. In above all four selected villages, direct contact to the fish farmers were done and information on the following matters were collected as per structured questionnaire.

## RESULT AND DISCUSSION

The primary data was collected from the four ox-bow lakes of Muzaffarpur district of Bihar and from Directorate of Fisheries, Govt of Bihar, Patna which represent different fisheries activities undergoing and resources available in the state. All these relevant data shows the present status of Fisheries in Bihar. The data during survey were presented in the form of tables and figures wherever necessary. Total no. of ponds and tanks of variable size is 40,520 which are distributed throughout the entire state covering a total water spread area of 68,821 ha comprising both government and private sector (2002-03). Total fish production in the state of Bihar is 2.61 lakh tonnes as against the total requirement of about 4.5 lakh tonnes per annum (2002-03, Up to March' 03). Per capita fish consumption in the state is one of the lowest in the country i.e., less than 1 kg/yr (Singh and Ahmad, 2003). Fishery development in ponds, tanks, reservoirs and lakes (Mauns and Chauras) are the major area under inland fishery development. These water areas can be very well used for inland fish production. Most of these areas produced fish much below their potential. In order to get consistent fish

yield through scientific management and also proper understanding of ecosystem is vital.

After studying the different aspects of fisheries in Bihar, it is apparent that the fish production from ponds and tanks at farmer's level managed by FFDA still remains at a low level of about 2275 kg/ha/yr. On the other hand, total catch of fish from reservoir is 18,217 i.e., 2.5 kg/ha/yr which is too less than the national average fish production from Indian reservoir (15 kg/ha/yr). The rate of fish yield has been reported by *Jha, 2004* to be at a desired level of 165-350 Kg/ha in flood plain lakes of Bihar and West Bengal. Though, the ecosystems are highly productive in nature. In the prevailing ecological condition, flood plain lakes in Bihar have a definite bearing on current yield pattern. There is a tremendous scope for fisheries development in flood plain lakes. Immediate adoption of scientific management species introduction, environmental and diversification of culture system. Effective and efficient management of resources, both physical as well as biological of resources both physical as well as biological holds the key for sustainable and environmental friendly fisheries development in flood-plain lakes.

The main resources concerned with the very low level of fish production from ponds, tanks, mauns, reservoirs etc. is the lack and knowledge of the farmers regarding scientific fish farming. During the survey it was noticed that most of the farmers are unfamiliar with the use of lime, fertilizer, feed with definite dose and time interval in the fish ponds. They simply stock the fish seed in the ponds in the name of fish culture followed by harvesting. As far as mauns is concerned very few farmers stock the fish seed and most of the farmers leave it on the mercy of nature for auto stocking. Most of the farmers don't know that Department of Fisheries provides training and subsidy of 25% both on variable and non-variable inputs. This may be attributed to the inert and village phobic attitude of Government of Fisheries officials towards fish farmers. Besides, extension network in the state is poorly developed. They often visit the fish farm and suggest solution for the problems. They never take feedback from farmers based on existing agro-climatic conditions at the farm for redressal either by themselves or by sending it to research centres. It is generally seen that Fisheries officials of the department of fisheries after undergoing training in Central Fisheries Institute like CIFE or CIFA on latest developing fish farming technology, often reveal or unable to explain by visiting at the farm of fish farmers and these cause it concern may be regarded as the stumbling block in the awareness of farmers towards scientific fish farming in Bihar as well as low fish production in ponds, tanks and mauns. Besides, at present the average production of fish seed is just around 350 million nos. (2002-03) against the requirement of about 600 million nos. per

annum. The major reason of concern is that from last few years the state department of fisheries has stopped fish seed production work on the ground that it has to be done by Fisheries Development Corporation. For this reason most of the departmental fish seed farms are now defunct. Unfortunately, the state Fisheries Development Corporation is presently not in position to cater to the fish seed requirement of the state due to absence of technical manpower. Presently the situation comes that farmers were fully dependent on river fish seed collected from rivers or the fish seed sold in the market comes from west Bengal, are mixed with spawns associated with weed as well as predatory fishes leading to mass mortality of fish seed after stocking in the pond. Also those farmers who manage to get hatchery produced seed, stock the seed in the pond without removing the weed and predatory fishes as well as aquatic weeds which really points the know-how of fish farming in Bihar.

In the state, there is very little bit of integration with livestock and particularly integration with piggery is not seen due to some religious feelings. Integration of fish with Makhana and trapa is one of the popular integrating system which is limited to north Bihar only. Though there is large scope of poultry integration in Bihar very few farmers adopted it due to lack of technical know-how. Also, the situation of Fisher Co-operative Society which is 370 in number in the state and is considered as a link between government and fish farmer and plays a major role in getting the different benefit and subsidy from government for both fish culture and socio-economic upliftment of fishery community has become bad to worst. Most of them have become functionless. Therefore, the government ponds, which are given on bid every year by Department of Fisheries, are generally taken by middleman persons due to their high approach and financially sound inspite of having provision of first priority to be given to Fishery Co-operative Society.

Besides after formations of Jharkhand state, the residual Bihar has lost a sizeable extent of water area

in the form of reservoir. At present only 30% of the total reservoir area in the residual Bihar covering water spread area of 7286 ha. But unfortunately only 11 medium and small reservoirs covering water spread area of 3589 ha have been taken for reservoir fisheries management by the Department of Fisheries, Govt. of Bihar. At present the average fish production from these reservoirs is about 2.5 Kg/ha/yr which is less than national average fish production in Indian reservoirs. In addition, inspite of having restriction on catching brooders during breeding season i.e., mid June to September, indiscriminate killing of the is continued without any check. Besides, there is no regulation on mesh size inspite of having rule in fishery legislation of the state that a net having zero mesh size is not permitted and this has played a crucial role in the extinction of many important food fishes which are essential in maintaining the food chain in reservoir.

Reservoir as well as mauns and chours inspite of having first priority to be given to Fishery Co-operative Society, at the time of bidding by Fishery Department officials, are generally taken by financially sound leader type person. A particular reservoir which comes into the hand of Fishery Co-operative Society is often seen that due to poor financial condition of the member, they are bound to sell their catch to the middleman and just get a minor share of the price what the consumer pay in the market. From the study it is revealed that the fish catch of the reservoir were mostly dominated by minnows which not only breeds profusely in the reservoir but also keenly competes with major carp in feeding. Since the minnows are effectively caught by drag net only, the fisherman should be encouraged to use these nets with greater frequency for eradication of minnows, particularly during reduced water level of summer months (February-June) in lotic sector, facilitated through availability of more suitable fishing areas. Similarly observation is reported by Desai and Shrivastava while working on Ecology of Fisheries of Ravishankar Sagar Reservoir, M.P (2004).

**Table 1.** District wise fish production from all sources with targeted production (2002-03 up to March' 03)

Name of district	Production target (in lakh tonnes)	Achievement (in lakh tonnes)
Madubani	0.1270	0.13
Darbhanga	0.1170	0.1294
Begusarai	0.1260	0.1220
Siwan	0.1060	0.1180
Saran	0.1060	0.1128
East Champaran	0.1060	0.1060
West Champaran	0.1060	0.1058
Muzaffarpur	0.1060	0.1058
Katihar	0.1060	0.1042
Vaishali	0.1060	0.1
Sitamarhi	0.10	0.0980
Nawada	0.0950	0.0901
Bhojpur	0.0850	0.0884
Saharsa	0.0850	0.0875

Purnia	0.0850	0.0850
Munger	0.0850	0.0836
Khagaria	0.0850	0.0810
Nalanda	0.0850	0.0810
Madhepura	0.0850	0.0800
Bhagalpur	0.0850	0.0750
Samastipur	0.1160	0.0630
Patna	0.0740	0.0612
Gopalganj	0.0740	0.0550
Rohtas	0.0530	0.0520
Buxar	0.0530	0.0516
Jehanabad	0.0515	0.05
Gaya	0.0635	0.0498
Auragabad	0.05	0.0427
Baka	0.0550	0.0425
Araria	0.0430	0.0384
Kissanganj	0.1060	0.1042
Jamui	0.0440	0.0303

**Table 2.** Details of quantity of fish landing and per capita income of fisher men.

Name of lake	Productive area/ha	No. of active fishermen	Quantity of fish landed	Present annual income of one (in Rs.)
Manika	108	210	5,088	727
Brahampura	45.5	100	2,502	751
Motipur	110	70	5,500	2,357
Kanti	100	125	4,700	1,128

**Note:** Per capita income were calculated based on 5 members in each family

**Table 3.** District wise fish seed production in Private and Govt. sector (2002-03)

Name of district	Fish seed production (in lakh)		Achievement (in lakh tonnes)
	Private	Govt.	
Madubani	191.00	--	--
Darbhanga	201.08	--	--
Begusarai	174.80		1.30
Siwan	86.00		4.00
Saran	90.50		2.00
East Champaran	177.25		2.25
West Champaran	160.00		2.87
Muzaffarpur	193.91		6.50
Katihar	149.79		--
Vaishali	127.00		2.35
Sitamarhi	115.00		--
Nawada	125.00		--
Bhojpur	67.88		--
Saharsa	141.00		--
Purnia	70.00		1.60
Munger	70.00		--
Khagaria	130.00		--
Nalanda	130		--
Madhepura	110.00		1.15
Bhagalpur	96.00		--
Samastipur	178.00		--
Patna	80.00		4.0
Gopalganj	50.00		--
Rohtas	140.00		--
Buxar	52.20		--
Jehanabad	85.00		--
Gaya	112.50		1.50

Auragabad	115.00	1.00
Banka	80.00	--
Araria	26.00	1.0
Kissanganj	39.00	--
Jamui	34.50	--

**Table 4.** Location of some fish seed hatchery in Bihar:

Name of district	Private/Govt. corporation	Production capacity (in million)	Present annual production of spawn (in million)
Danapur(Patna)	Fish development corporation	150	95
Sitamarhi	Fish development corporation	150	190
Madhubani	Fish development corporation	40	--
Mangalgarh(Sitamarhi)	Pvt.	25	11
Mahawa(East Champaran)	Pvt.	80	27
Matasya priya Udyog (East Champaran)	Pvt.	90	31
Chhoti Dilahi	Pvt.	30	5.0
Yadavpur (Gopalganj)	Pvt.	30	--
Motipur (Muzaffarpur)	Pvt.	25	2.0
Kaimur	Govt. (under construction)	--	--

**Table 5.** Details of distribution of floodplain wetlands in India

State	Distribution(districtwise)	River basin	Local name	Area (ha)
Arunachal Pradesh	East Kameng, Lower Subansiri, East Siang, Dibang Valley, Lohit, Tirap and Changlang	Kameng, Subansiri, Dibang, Lohit Dihing Tirap	Beel	2,500
Assam	Brahmaputra and Barak valley district	Brahmaputra and Barak	Beel	10,000
Bihar	Saran, Champaran, Saharsa, Muzaffarpur, Darbhanga, Munger and Purnea	Gandak and Koshi and Dhar	Maun, Chaur	40,000
Manipur	Imphal, Thaubal and Bishnupur	Iral, Imphal and Thaubal	Pat	16,500
Meghalaya	West Khasi hills and East Karo hills	Somehwari and Jinjiram	Beel	213
Tripura	North, South and West Tripura district	Gumti	Beel	500
West Bengal	24-Praganas north and South, Hooghly, Nadia, Malda, murshidabad, Maldah, CoochBihar, Burdawan, North and South Dinajpur and Midnapur	Hooghly, Ichamati, Bhagirathi, Chumi, Kalindi,Dharub, Dharala, Pagla, Jalangi, Behula, Torsa and Mahananda	Eel	42,500
Total			Charah and Baor	202,213

**Table 6.** Distribution and area of existing of Ox-bow lake in Gandak basin of Muzaffarpur district.

Name of district	Area in ha
Brahmpura	45.50
Manika	105.50
Motipur	110.00
Kanti	100.00

Jhapaha	140.00
Murra	15.00
Rahuwa	30.00
Bhoosra	45.00
Bachaha	30.00
Semera	16.00
Matiha	20.00
Rajwara	12.00
Morsandi	60.00
Ghosod	50.00
<b>Total</b>	<b>779</b>

Source : Department of Fisheries, Muzaffarpur, Bihar

**Table 7.** Details of reservoirs in different district of Bihar

Name of district	Name of the reservoirs	Type of reservoirs (Small : <1000 ha, Medium : 1000-5000 ha, Large : 5000-10,000 ha)	Area (in ha)
Banka	Badua Jalsay	Medium	1335.50
	Amahara Jalsay	Small	21.00
	Madhyagiri Jalsay	Small	269.00
Bhagalpur	Belharna Jalsay	Small	20.00
	Chandan Jalsay	Medium	1050.00
	Jalkund	Small	5.00
Munger	Kharagpur Jalsay	Small	210.00
	Morvey Jalsay	Small	40.50
Jamui	Nagi Jalsay	Small	439.00
	Amrit Jalsay	Small	20.00
	Nakti Jalsay	Small	179.00
Total			3,589.00

Source: Statistical division of Directorate of Fisheries, Govt. of Bihar

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## CYTOMORPHOLOGICAL CHARACTERIZATION OF *OCIMUM BASILICUM* AND *O. TENUIFLORUM* GERMPLASM

Aditi Saha\*

Department of Botany, NarasinhaDutt College, Howrah 711101

Email: sahaaditi2007@rediffmail.com

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**Abstract:** *Ocimumbasilicum* (sweet basil) and *O. tenuiflorum* (holy basil) of the genus *Ocimum* (basil; Family: Labiatae) obtained from Medicinal Plant Garden, Narendrapur are grown in the experimental field plots of Kalyani University (West Bengal plains) and cytotaxonomically characterized. Quantitative estimation of essential oil content from leaves and flower tops are also performed. *O. basilicum* and *O. tenuiflorum* show  $2n=72$  and  $2n=36$  respectively in meiocytes. The objective of the work is to catalogue the germplasms of the species under study for proper maintenance of genetic stock(s) for future exploration.

**Keywords:** Basil, Meiosis, Taxonomic characterization, Essential oil, Genetic stock

### INTRODUCTION

*Ocimumbasilicum* (sweet basil) and *O. tenuiflorum* (holy basil) of the genus *Ocimum* (basil; Family: Labiatae) are important source of essential oil (methyl chavicol, eugenol, linalool, camphor and cinnamate – Simon *et al.* 1990; extracted from leaves and flowering tops) apart from possessing immense therapeutic uses (Prakash and Gupta 2005, Datta *et al.* 2010). The essential oil of basil possesses anti-cancerous property (Aruna and Sivaramakrishnan 1996) apart from having insecticidal (Chogo and Crank 1981, Chavan and Nikam 1982) and nematocidal (Chatterjee *et al.* 1982) activities. *O. tenuiflorum* is designated as ‘elixir of life’ in Ayurveda as it is believed to promote longevity (Puri 2002). Considering the significance, the species should be under sustainable cultivation with proper characterization of the germplasm under study. Such study can be helpful for maintenance of genetic stock(s). With a view to it, present study characterized *Ocimumbasilicum* and *O. tenuiflorum* germplasms cytologically that are grown in Kalyani University campus (Nadia, West Bengal plains). Quantitative assessment of essential oil content (from leaves and flowering tops) of the germplasms is also performed.

### MATERIAL AND METHOD

#### Germplasm

Mother seed stock of *Ocimumbasilicum* L. (moisture content - 8.62%) and *O. tenuiflorum* L. purple type (moisture content - 6.50%) were procured from Medicinal Plant Garden, Narendrapur, Ramkrishna Mission (Voucher specimens deposited in the

Herbarium, Botany Department, Kalyani University), West Bengal, India. Seeds of both species were sown in late November in the experimental field plots of Kalyani University (West Bengal plains, latitude: 22°50' to 24°11' N and longitude: 88°09' to 88°48' E, altitude: 9.75 m; sandy loamy soil, pH: 6.85) to raise plants.

#### Taxonomical studies

The mature plants of both species were examined and the study includes detail description of every part of the species with Olympus dissecting microscope under 10X. Measurements of the leaves, floral parts and seeds were made. On an average 15 seeds were measured in either of the species in a Stereo dissecting microscope. 100 seed weight and moisture content were also determined.

#### Meiosis

Flower buds of suitable sizes from 5 randomly selected plants (for each species) plants of *O. basilicum* and *O. tenuiflorum* were fixed in Carnoy's fluid (4 to 5 p.m.) and two changes were given in the fixative at an interval of 24h. Anthers were squashed in 2% aceto-carmin solution and well scattered PMCs were scored at metaphase I (MI) and anaphase I (AI). Meiotic analysis was performed from 3 sites (5 plants assessed in each site for both species; data pooled over the plants in each site) in the field to assess variation, if any. Photomicrographs were taken from suitable preparation. Pollen grains from mature buds were also stained in 2% aceto-carmin and uniformly stained pollen grains were considered fertile (Marks 1954).

#### Acetolysis studies

Acetolysis technique was adopted as per Erdtman (1952) to study the shape, size and ornamentation of the pollen grains in both species.

\*Corresponding Author

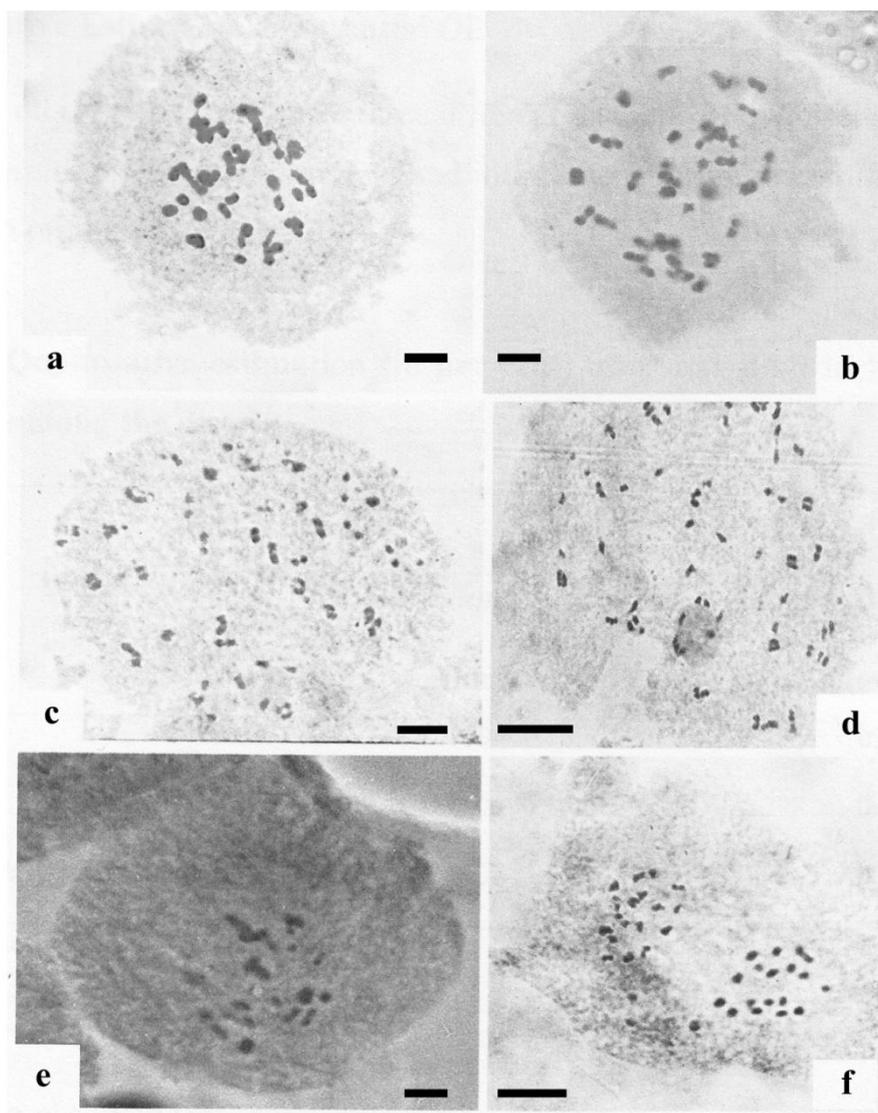
**Table 1.** Metaphase I configurations of two species of *Ocimum*

District	<i>O. basilicum</i> (2n=72)			<i>O. tenuiflorum</i> (2n=36)		
	PMCs scored	II	I	PMCs scored	II	I
Site I	172	35.40	1.20	192	17.20	1.60
Site II	198	35.12	1.76	186	17.72	0.56
Site III	166	35.64	0.72	174	17.92	0.16
$\chi^2$ test of heterogeneity probability		>0.05	<0.05		>0.05	<0.05

**Table 2.** Quantitative estimation (in per cent) of essential oil in two species of *Ocimum*

District	Genotypes			
	<i>O. basilicum</i>		<i>O. tenuiflorum</i>	
	Leaf	Inflorescence	Leaf	Inflorescence
Site I	0.380-0.412	0.180-0.182	0.312-0.315	0.241-0.246
Site II	0.391-0.402	0.160-0.168	0.290-0.298	0.224-0.229
Site III	0.355-0.371	0.160-0.169	0.301-0.306	0.191-0.199
$\chi^2$ test of heterogeneity probability	>0.05	>0.05	>0.05	>0.05

**Figure plate 1 (a-b)** Plant types of *Ocimum* spp. (a) *Ocimum basilicum* and (b) *Ocimum tenuiflorum*.



**Figure plate 2 (a-f)** showing meiotic configuration of *Ocimum basilicum* (a-d) and *O. tenuiflorum* (e-f). (Fig a-d) MI showing a -36II, b-33II+6I, c-30II +12I, d-29II+14I; e- MI showing 18II, f- AI with 18:18 separation scale bar = 2  $\mu$ m

## RESULT AND DISCUSSION

### Taxonomical characterization

Taxonomical description of the plant species of *Ocimum* are given below

#### *O. basilicum* (Fig. 1a)

Annual branched herb, 66.6 cm high, 4 angular, hairy throughout, prominent along the 4-angles or ridges of the stem, solid, green to dull green, leaves opposite-decussate, simple, lanceolate to ovate lanceolate, or broadly elliptic, 2 to 3 cm long, and 1.5 cm wide, acute, margin serrate-dentate, base cuneate, venation unicostate, 3-4 pairs of alternate secondary, hairy on both surfaces, gland dotted, dull green petiolate, petioles 1 cm long, hairy; inflorescence with about verticillasters, mostly 6 flowers in a whorl, 3 to 5 in each axis of the bract, finally forming spikes of 30 cm long, inflorescence bract elliptic, 7 mm long and

2.5 mm wide, purple-copper coloured, long hairy, hairs silky, midvein distinct; flowers complete, bisexual, hypogynous, 2 lipped, white, pedicellate, pedicels slender 4-angular, 1.2 to 2 cm long, purple, short hairy; calyx gamosepalous, 1.6 to 1.8 mm long, united part of 6 to 8 mm, free lobes 8 to 10 mm long, posterior sepal broadly ovate, 5.5 to 8 mm long and 10 mm wide, hairy on the outer surface, more acute, long hairy, greenish purple, smaller than the posterior sepal and the anterior sepals, anterior sepals triangular-dentate 8 to 14 mm long and 6 mm wide, acute to acuminate, long hairy, purple-green, longer than the lateral calyx lobes, base of the calyx tube on the posterior end just above the pedicel tumid; corolla 2-lipped, tubular part 1.73 to 2.22 mm long, glabrous both within and without, light pinkish white, posterior lobe oblong, shallowly 2-lobed at apex, 2.4 to 3.2 mm long and 1.4 mm wide, glabrous,

all white; stamens 4, didynamous, filaments of posterior-lateral pairs long slender, 3.4 to 3.9 mm long, attached above the base, not bifurcate not dentate, white, glabrous, anthers 2-celled, ovoid, dorsifixed, dehiscence longitudinal, latrorse, orange, glabrous; carpels 2, syncarpous, ovary ellipsoid, inconspicuously 4-lobed, 0.4 to 0.6 mm across glabrous, style 6.4 to 7.6 mm long, pinkish to deep pinkish on the upper end, glabrous, stigma bifid, pinkish, glabrous.

#### *O. tenuiflorum* (Fig. 1b)

Annual to perennial herb, branched, hairy; leaves opposite decussate, ovate to oblong, 4.3 to 5 cm long, and 3.1 cm wide, apex rounded, margin serrate with setae on serrate end, base rounded venation unicostate reticulate with 4(5) pairs of pinnate secondaries, basal 2 pairs opposite, 3<sup>rd</sup> pair sub-opposite 4(5th) pair alternate, arched and united with the surface adjacent secondary in the intramarginal region (brochidodromous), distinct on the lower surface, veins purple to deep purple above, major veins depressed above, plumose hairy on both surfaces, gland-dotted below, greenishpurple above, dull green beneath, petiolate, petioles slender 8 to 10 mm long, slightly ridged, ciliate, hairs unicellular, woolly white, greenish with slight purple shade; inflorescence with primary rachis, 7.2 cm long with 11 whorls (verticels) of 6 flowers each, secondary rachis opposite decussate, 3 to 4.5 cm, 5 to 7 verticels, 6 flowered or rarely less in number, inflorescence bract 2, ovate, 2.5 mm long and 2 mm wide, apex acute margin ciliate, entire, base cordate, rounded or rarely sub-truncated, surface pubescent more on the lower surface, purple green, later brownish, venation 3-veined, 2 basal inconspicuous; flowers complete, bisexual, hypogynous, zygomorphic, pentamerous white with slightly pink, ebracteate, pedicellate, pedicels long slender, 2.6 to 3 cm long, arched and curved, purple, hairy; calyx gamosepalous, 2.4 to 3 mm long, bilobed; sepals 5, trimorphic, posterior one broadly ovate or broadly orbicular, 1.9 mm wide, apex apiculate, entire, hairy on the outer surface and margin, accrescent in fruit, lateral 2 sepals dentate, 2 mm long, purple, hairy, anterior 2 sepals dentate to acuminate, 2.5 mm long, purple, hairy, tubular part 1.5 mm long; corollagamopetalous, bilabiate, 5 mm long, tubular part 2.2 mm, petals oblong, 1.7 to 2.5 mm long, rounded, white with slightly pinkish, posterior to larger than the lateral and anterior; stamens 4, didynamous (2+2), filaments long slender to filiform, 3.8 to 4.5 mm long, epipetalous, anthers 2-celled, oblong ovoid, dorsifixed, dehiscence longitudinal, dull yellowish, glabrous; carpels 2, syncarpous, ovary superior, 4-lobed, 0.3 to 0.5 mm long, style slender, about 5.5 mm long pinkish white, glabrous.

#### Meiotic analysis

##### *O. basilicum* (Fig. 2a-d)

PMC squashes showed  $2n=72$  chromosomes in all cases. The plants formed 36II in metaphase I cells

(Table 1). PMCs with 35II+2I, 34II+4I, 33II+6I, 32II+8I and 31II+10I were also found. Bivalent frequency among the plants varied from 35.12 to 35.64 per cell and those bivalents (random distribution evidenced from  $\chi^2$  test of heterogeneity  $\chi^2=0.265$  at 9 DF,  $p>0.95$ ) tended to form variable groups at MI; although univalent range: from 0.72 to 1.76/cell (non-randomly distributed,  $\chi^2=21.27$  at 9 DF,  $p<0.01$ ) were often associated in groups with bivalent. AI segregation of chromosome is mostly balanced and rarely cell from laggards varied from 1-4/PMC. Pollen fertility among the samples ranges between 46.85% and 53.12%.

##### *O. tenuiflorum* (Fig. 2e-f)

The species had  $2n=36$  chromosomes in the meiocytes always (Table 1) and range from 17.20 to 17.92 (random distribution,  $\chi^2=0.504$  at 9 DF,  $p>0.95$ ). Univalent per cell is found to vary 0.16 to 1.60 (non-randomly distributed,  $\chi^2=104.96$  at 9 DF,  $p<0.01$ ) at MI. while the rest had 17II+2I, 16II+4I, 33II+6I and 13II+10I. AI is cytologically (18:18) balanced with occasional formation of laggards; bridges and cells with unequal separation of chromosomes. Pollen fertility was also noted.

Present investigation reports  $2n=72$  chromosomes in *O. basilicum* which is rather contrary to  $2n=16$  (SZ Borsos 1970) and  $2n=48$  (Morton 1962, Mehera and Gill 1972, Puspangadan *et al.* 1975, Sanjappa 1979, Puspangadan and Sobti 1982); however the number  $2n=72$  has earlier been reported in *Ocimum* for the species *O. americanum* (Puspangadan and Sobti 1982). Critical analysis of taxonomic data of the genus *Ocimum* is provided that *O. americanum* is a synonym of *O. basilicum* L. (Morton 1962, Banerjee and Maity 2000). *O. tenuiflorum* has  $2n=36$  chromosomes always in meiocytes. Bir and Sahoo (1980) and Singh (1980) also reported  $2n=36$  chromosomes for the species but it also stated to be as  $n=16$  (Mehera and Gill 1972, Khosla and Sobti 1985),  $n=16+0-3B$  (Vij and Kashyap 1976),  $n=17$  (Singh 1980) and 32 (Tischler 1938).

#### Pollen morphology

##### *O. basilicum*

Average pollen size  $75.68\mu \times 75.32\mu$  (ranges:  $68.00\mu \times 68.00\mu$  to  $80.00\mu \times 80.00\mu$ ); spheroidal in shape; zonocolpate, colpi 6, deep; surface with small reticulation, uniform, pentagonal rarely hexagonal, super reticulate; wall structure-tectate columellate, collumellae numerous, distinct.

##### *O. tenuiflorum*

Average pollen size  $32.49\mu \times 29.58\mu$  (ranges:  $29.58\mu \times 26.10\mu$  to  $34.80\mu \times 33.10\mu$ ); spheroidal to oval; zonocolpate, colpi 6, deep; surface reticulate, reticulum small with often irregular size and shape; uniform, wall structure-tectate columellate, collumellae numerous, distinct.

#### Bio-chemical analysis

##### Quantitative estimation of essential oil

Essential oil content analysed for three different districts following steam distillation in two species of

*Ocimum* from leaf and inflorescence have been presented in table 2.

It has been observed that there is almost no difference in oil content of two species of *Ocimum* among these three districts.

## CONCLUSION

The study is significant for proper indexing of the germplasms under study that can act as an important genetic resource in sustainable cultivation.

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## EFFECT OF TIME OF AIR LAYERING AND IBA CONCENTRATIONS ON THE ROOTING BEHAVIOUR OF PANT PRABHAT GUAVA (*PSIDIUM GUAJAVA* L.) UNDER SUB-TROPICAL CONDITION OF GARHWAL HIMALAYA

Dinesh Chandra Naithani<sup>1\*</sup>, Anant Ram Nautiyal<sup>2</sup>, Deepak Kumar Rana<sup>1</sup> and Deepak Mewar<sup>1</sup>

<sup>1</sup>Department of Horticulture, School of Agriculture and Allied Science, H.N.B. Garhwal University (A Central University), Srinagar Garhwal, Uttarakhand, India-246174

<sup>2</sup>High Altitude Plant Physiology Research Centre (HAPPRC), School of Agriculture and Allied Science, H.N.B. Garhwal University (A Central University), Srinagar Garhwal, Uttarakhand, India-246174

Email: [naithani.dinesh@yahoo.com](mailto:naithani.dinesh@yahoo.com)

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**Abstract:** The present study were undertaken at the Orchard Section, Horticultural Research Centre and Department of Horticulture, Chauras Campus, School of Agriculture and Allied Science, HNB Garhwal University (A Central University), Srinagar Garhwal, Uttarakhand, India during the rainy season of the year 2016 to study the effect of time of air layering and IBA concentrations on the rooting behaviour of Pant Prabhat Guava (*Psidium guajava* L.). The experimental findings showed that the minimum days taken to root appearance (28.40days), maximum rooting percentage (92.20%), maximum number of roots per layer (24.80), maximum length of longest root per layer (14.20cm), maximum diameter of thickest root per layer (1.86mm) and maximum percentage of layers showing secondary roots (70.00%) were significantly superior when layering was done on 15<sup>th</sup> July and treated with 4500 ppm concentration of IBA.

**Keywords:** IBA, Rooting, Layering, Secondary roots

### INTRODUCTION

Guava (*Psidium guajava*), the apple of the tropics belongs to the family myrtaceae, and is one of the most common fruits in India. It claims to be the fifth most important fruit in area and production after banana, mango, citrus and papaya. Guava is quite hardy, prolific bearer and highly remunerative even without much care. The genus *Psidium* contains 150 species, most of which are fruit bearing trees. The basic chromosome number of guava is 11. Most of cultivars are diploid (2n=22), but some natural and artificial triploids (3n=33), that generally produce seedless fruits (Jaiswal, , 1992). In Uttarakhand, major guava producing areas are in Pithoragarh, Udham Singh Nagar, Haridwar, Dehradun, Pauri, Tehri and Uttarkashi districts. Guava is propagated by seeds and vegetative methods. Seed propagated plants start bearing fruits in 6-8 years, while those from vegetative method (air layering) start bearing in 2-3 years of age. Seedling trees exhibit lot of variations in quality of fruits and require more area for space in orchard due to the more vigorous growth habit. The propagation of guava through seeds is not encouraged because the seedlings have long juvenile phase, give lower yields and bear poor quality fruits. To maintain true-to-type cultivar, it is necessary to go through the vegetative method of propagation for guava (Bose *et al.*, 1986). Propagation of guava by air-layering is done during summer of the year i.e. from February to August with varying success and survivability. Time of layering and detachment of layers from the mother plants are the important

factors for rooting success because of presence of sufficient soil moisture, humidity and optimum temperature which are prerequisites of maximum survival of the detached air-layers. Kanwar and Khalon (1986) reported that layering was successful when carried out between mid July and early October in India. BARI (2002) reported that layers prepared in mid June showed the best performance of rooting percentage, survivability and growth of air-layers. There are many factors which affect the propagation of a particular plant species through layering. These factors include the physiological condition of the mother plant, nutrient and water supply, etiolation, age of mother plant, growing media, wrapping material, time/season of operation, external environmental conditions and application of root inducing substances. Among all these factors time of operation and application of different concentration of root inducing/promoting substances play an important role in the propagation of plants by air-layering. Hence, the present investigation was conducted to study the effect of time of air-layering and IBA concentration on the rooting behaviour of Pant Prabhat Guava (*Psidium guajava* L.)

### MATERIAL AND METHOD

#### Detail of Experiment

The experiment was conducted under the open field condition at orchard section, Horticultural Research Centre and Department of Horticulture, Chauras Campus, School of Agriculture and Allied Science, HNB Garhwal University (A Central University)

\*Corresponding Author

Srinagar Garhwal, Uttarakahnd, India during the rainy season of the year 2016. The experimental detail is follows-

Time of air-layering (T)		IBA Concentration(C)	
Notation	Treatments	Notation	Treatments
(T <sub>1</sub> )	15 <sup>th</sup> June	(C <sub>1</sub> )	IBA @ 1500 ppm
(T <sub>2</sub> )	30 <sup>th</sup> June	(C <sub>2</sub> )	IBA @ 3000 ppm
(T <sub>3</sub> )	15 <sup>th</sup> July	(C <sub>3</sub> )	IBA @ 4500 ppm
(T <sub>4</sub> )	30 <sup>th</sup> July	(C <sub>0</sub> )	Control

**Treatment Combination**

Notation	Treatments
T <sub>1</sub> C <sub>1</sub>	15 <sup>th</sup> June+IBA @ 1500 ppm
T <sub>1</sub> C <sub>2</sub>	15 <sup>th</sup> June+IBA @ 3000 ppm
T <sub>1</sub> C <sub>3</sub>	15 <sup>th</sup> June+IBA @ 4500 ppm
T <sub>1</sub> C <sub>0</sub>	15 <sup>th</sup> June+IBA @ 0 ppm
T <sub>2</sub> C <sub>1</sub>	30 <sup>th</sup> June+IBA @ 1500 ppm
T <sub>2</sub> C <sub>2</sub>	30 <sup>th</sup> June+IBA @ 3000 ppm
T <sub>2</sub> C <sub>3</sub>	30 <sup>th</sup> June+IBA @ 4500 ppm
T <sub>2</sub> C <sub>0</sub>	30 <sup>th</sup> June+IBA @ 0 ppm
T <sub>3</sub> C <sub>1</sub>	15 <sup>th</sup> July+IBA @ 1500 ppm
T <sub>3</sub> C <sub>2</sub>	15 <sup>th</sup> July+IBA @ 3000 ppm
T <sub>3</sub> C <sub>3</sub>	15 <sup>th</sup> July+IBA @ 4500 ppm
T <sub>3</sub> C <sub>0</sub>	15 <sup>th</sup> July+IBA @ 0 ppm
T <sub>4</sub> C <sub>1</sub>	30 <sup>th</sup> July+IBA @ 1500 ppm
T <sub>4</sub> C <sub>2</sub>	30 <sup>th</sup> July+IBA @ 3000 ppm
T <sub>4</sub> C <sub>3</sub>	30 <sup>th</sup> July+IBA @ 4500 ppm
T <sub>4</sub> C <sub>0</sub>	30 <sup>th</sup> July+IBA @ 0 ppm

**Cultivar** Pant Prabhat  
**Experimental design** Randomized Block Design  
**Number of replications per treatment** 3  
**Number of air layers per treatment** 10  
**Number of treatments** 16  
**Total number of air layers** 3x10x16 = 480

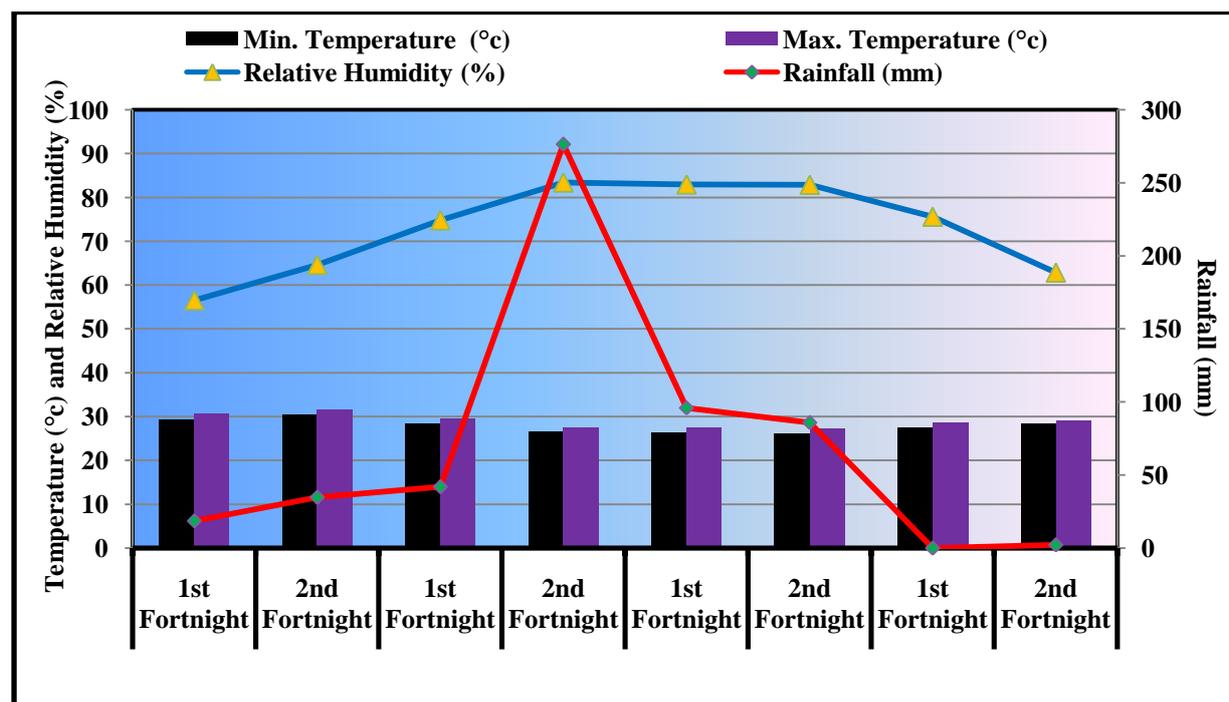


Fig. 1: Fortnightly meteorological data during the experimental period (June-September) 2016

### Materials Used for the Experiment

Guava trees of the cultivar Pant Prabhat were selected for performing air layering. Clear polythene sheet (size: 20×20cm), sharp knife, thread (sutli), rooting media (Sphagnum moss), IBA, mother plant and sharpening stone were used.

### Preparation of IBA Solution

For the preparation of one liter stock solution of 4500 ppm concentration of IBA, 4500 mg IBA (4.5gm) was weighted and it was then dissolved in small amount of alcohol and few drops of ammonium hydroxide were also added to stop precipitation. Further, it was diluted to one liter with distilled water and stored in cool and dark place. The working solutions of required concentrations, viz; 1500 ppm, 3000 ppm and 4500 ppm were prepared from this stock solution.

### Selection of Branches

For guava air-layering healthy, well matured, uniform, disease free, vigorously growing lateral shoots having green and brown portion at the apical and basal portion respectively of past season growth with approximate diameter of 1.0cm and length of 30cm were selected. Careful observations were also made to ensure that the selected branches had sufficient leaves on them.

### Technique Followed to Perform Air Layering

On dated 15<sup>th</sup> June, 30<sup>th</sup> June, 15<sup>th</sup> July and 30<sup>th</sup> July, of 2016 air layering was done on the selected shoots by removing a strip of bark (phloem) 2.0 to 2.5 cm wide just below the bud by giving two circular cuts about 30-35cm below from shoot tip and then the exposed portion was rubbed without causing any injury to the xylem with the help of a knife. Then the upper portion of exposed shoots was sprayed with different concentrations of IBA according to the treatments. The exposed wood with two centimeters above and below portions was then covered with sphagnum moss soaked overnight in water. The control shoots were treated with only sphagnum moss soaked overnight in water. To cover the rooting medium completely a piece of clear polythene sheet (size: 20×20cm) was wrapped. The two ends of the wrapping material were carefully tied up thoroughly with thread and left for rooting.

### Detachment of Air Layers

The layers were separated from the mother plants 60 days after layering operation with the help of secateurs when the outer surface of rooting medium within the wrapped polythene sheet was full of newly formed roots.

### Observations Recorded

The following observations on rooting behaviour were recorded during the period of experimentation.

### Days taken to root appearance

Air-layers were visited regularly and days taken to first root appearance were counted under each treatment within each replication from the date of

air-layering operation. Then mean days required for root appearance were calculated.

### Rooting percentage

The number of successful layers was counted after 60 days from the date of operation and the result was calculated in percentage by using the following formula-

$$\frac{\text{Number of successful rooted layers in a treatment}}{\text{Number of layers done in a treatment}} \times 100$$

### Number of roots per layer

Three randomly selected rooted layers from each replication were taken for recording the data on the number of roots per layer. Mean number of roots per layer was calculated by dividing the total number of roots, with the number of layers.

### Length of the longest root per layer (cm)

This observation was recorded after the detachment of air-layers (60 days after operation). Three randomly selected rooted layers from each replication were taken for recording the data on the length of the longest root. Length of longest root in each layer was measured with the help of measuring scale and mean was calculated.

### Diameter of the thickest root per layer (mm)

This observation was recorded after the detachment of air-layers (60 days after operation). Three randomly selected rooted layers from each replication were taken for recording the data on the diameter of the thickest root.

### Percentage of layers showing secondary roots (%)

The percentage of layers showing secondary roots was recorded in each treatment 60 days after detachment of air-layers from mother plant. It was calculated with the help of following formula:

$$\frac{\text{Number of layers showing secondary roots in a treatment}}{\text{Number of layers done in a treatment}} \times 100$$

### Statistical analysis

Data recorded during the course of investigations were subjected to statistical analysis under randomized block design as described by Snedecar and Cochran (1987). Valid conciliations were drawn after the determination of significance of difference between the treatments, at 5 per cent level of probability. Critical difference was calculated in order to compare the treatment means.

## RESULT AND DISCUSSION

The results obtained on days taken to root appearance, rooting percentage, number of roots per layer, length of the longest root per layer, diameter of the thickest root per layer and percentage of layers showing secondary roots are shown in table 1.

**Days taken to root appearance:** Time of air layering and IBA concentrations significantly affected the days taken to root appearance in Pant

Prabhat guava as shown in Table 1. Among all treatments, minimum days taken to root appearance (28.40days) on air layering of guava was recorded under T<sub>3</sub>C<sub>3</sub> (15<sup>th</sup> July+IBA @ 4500 ppm) treatment while maximum days taken to root appearance (54.50days) was observed under T<sub>1</sub>C<sub>0</sub> (15<sup>th</sup> June+IBA @ 0 ppm) treatment. The decreased number of days taken to root initiation could be due to the appropriate environmental conditions viz. temperature, rainfall and humidity (Fig. 1) with increased concentration of IBA. Tayade *et al.* (2017) also reported minimum days taken to root appearance in layers of pomegranate when layering operation performed in July month. These findings are more or less similar to the observations reported by Reang *et al.* (2016) in jackfruit, Baghel *et al.* (2016) in guava cv. L-49, Manga *et al.* (2017) in guava cv. Sardar.

**Rooting percentage:** Results shown in Table 1 revealed that T<sub>3</sub>C<sub>3</sub> (15<sup>th</sup> July+IBA @ 4500 ppm) treatment found best in relation to rooting percentage (92.20%), whereas least rooting percentage (40.00%) was found under the treatment T<sub>2</sub>C<sub>0</sub> (30<sup>th</sup> June+IBA @ 0 ppm). Increased concentration of IBA may have caused mobilization and utilization of carbohydrates and nitrogen fraction with the presence of co-factors at wound (griddled) site which may have helped in better root initiation coupled with appropriate layering time (Singh and Mahato, 2016). Hence, IBA at highest concentration (4500 ppm) resulted in highest rooting percentage of the guava air layers. Rooting percentage also depends on the physiological condition of plant. Tayade *et al.* (2017) also reported maximum rooting percentage of air layers of pomegranate when layering was done in July month. These findings are more or less in conformity with the results reported by Bhagat *et al.* (1998, 1999a, 1999b) in guava, Baghel *et al.* (2016) in guava cv. L-49, Manga *et al.* (2017) in guava cv. Sardar, Singh and Mahato in guava (2016) in guava, Tomar (2011) in jackfruit and Tomar (2016) in *Spondias pinnata*.

**Number of roots per layer:** The data pertaining to number of roots per layer (Table 1) shows that 15<sup>th</sup> July air layering time and higher IBA concentration have significant effect on number of roots per layer. Maximum number of roots per layer (24.80) was recorded in layers of T<sub>3</sub>C<sub>3</sub> (15<sup>th</sup> July+IBA @ 4500 ppm) treatment whereas minimum number of roots (3.74) was recorded under T<sub>1</sub>C<sub>0</sub> (15<sup>th</sup> June+IBA @ 0 ppm) treatment. IBA has been found to be most effective in producing maximum number of roots with better vigour. Similar trend was observed by Bhagat *et al.* (1998, 1999a, 1999b) in guava, Baghel *et al.* (2016) in guava cv. L-49, Reang *et al.* (2016) in jackfruit, Manga *et al.* (2017) in guava cv. Sardar,

Singh and Mahato (2016) in guava, Tomar (2011) in jackfruit and Tomar (2016) in *Spondias pinnata*.

**Length of the longest root per layer:** The data on length of the longest root per layer presented in Table 1 revealed that length of the longest root was found significant in respect to time of air layering and IBA concentrations. The treatment T<sub>3</sub>C<sub>3</sub> (15<sup>th</sup> July+IBA @ 4500 ppm) was found to have maximum length of longest root per layer (14.20cm) whereas minimum length of the longest root per layer (3.40cm) was observed under the treatment T<sub>1</sub>C<sub>0</sub> (15<sup>th</sup> June+IBA @ 0 ppm). Tayade *et al.* (2017) also found increased length of primary roots in air layers of pomegranate performed in the month of July. Many workers have reported successful results by the use of plant growth regulators in stimulating the root length in air layering of guava crop (Bhagat *et al.*, 1999b; Singh and Bhuj, 2000; Tyagi and Patel, 2004 and Singh *et al.*, 2007). Similar trends were also found by Bhagat *et al.* (1998, 1999a) in guava, Baghel *et al.* (2016) in guava cv. L-49, Manga *et al.* (2017) in guava cv. Sardar, Singh and Mahato (2016) in guava, Tomar (2011) in jackfruit and Tomar (2016) in *Spondias pinnata*.

**Diameter of the thickest root per layer:** The data presented in Table 1 indicate that all the treatments of time of air layering and IBA concentrations were found to vary significantly in terms of diameter of roots. The maximum diameter of the thickest root per layer (1.86mm) was recorded in layers of T<sub>3</sub>C<sub>3</sub> (15<sup>th</sup> July+IBA @ 4500 ppm) treatment, whereas minimum diameter of the thickest root per layer (1.11mm) was noticed in layers of T<sub>1</sub>C<sub>0</sub> (15<sup>th</sup> June+IBA @ 0 ppm) treatment. This result is in more or less conformity with the findings of Chauhan (2012) in fig, Chawla *et al.* (2012) in litchi, Tomar (2011) in jackfruit and Udhavrao *et al.* (2017) in pomegranate.

**Percentage of layers showing secondary roots:** Among all the treatments, percentage of layers showing secondary roots was recorded maximum (70.00%) under the treatment T<sub>3</sub>C<sub>3</sub> (15<sup>th</sup> July+IBA @ 4500 ppm), whereas the air layers in [T<sub>1</sub>C<sub>0</sub> (15<sup>th</sup> June+IBA @ 0 ppm) and T<sub>2</sub>C<sub>0</sub> (30<sup>th</sup> June+IBA @ 0 ppm) treatments] failed to produce secondary roots. The minimum percentage of layers showing secondary roots (3.33) were recorded under treatments (T<sub>4</sub>C<sub>0</sub> (30<sup>th</sup> July+IBA @ 0 ppm)). Tomar (2011) in jackfruit also reported maximum percentage of layers showing secondary roots when layering was performed during 25-26<sup>th</sup> July and treated with 10000 ppm IBA. These findings shows that time of air layering operation and increased concentration of growth regulator is beneficial for secondary roots development in air layering of guava.

**Table 1.** Effect of Time of Air Layering and IBA Concentrations on the Rooting Behaviour of Pant Prabhat Guava (*Psidium guajava* L.)

Treatments	Days taken to root appearance	Rooting percentage (%)	Number of roots per layers	Length of longest root per layer (cm)	Diameter of thickest root per layers (mm)	Percentage of layers showing secondary roots (%)
T <sub>1</sub> C <sub>1</sub> (15 <sup>th</sup> June+IBA @ 1500 ppm)	47.00	56.67	7.74	6.50	1.34	7.78
T <sub>1</sub> C <sub>2</sub> (15 <sup>th</sup> June+IBA @ 3000 ppm)	39.00	64.44	13.00	8.34	1.48	31.10
T <sub>1</sub> C <sub>3</sub> (15 <sup>th</sup> June+IBA @ 4500 ppm)	34.30	75.56	15.30	10.50	1.58	46.70
T <sub>1</sub> C <sub>0</sub> (15 <sup>th</sup> June+IBA @ 0 ppm)	54.50	41.11	3.74	3.40	1.11	0.00
T <sub>2</sub> C <sub>1</sub> (30 <sup>th</sup> June+IBA @ 1500 ppm)	44.20	42.22	8.52	7.84	1.30	20.00
T <sub>2</sub> C <sub>2</sub> (30 <sup>th</sup> June+IBA @ 3000 ppm)	39.00	53.30	13.90	10.10	1.41	27.80
T <sub>2</sub> C <sub>3</sub> (30 <sup>th</sup> June+IBA @ 4500 ppm)	31.80	71.10	17.30	12.60	1.54	52.20
T <sub>2</sub> C <sub>0</sub> (30 <sup>th</sup> June+IBA @ 0 ppm)	50.60	40.00	5.00	3.73	1.14	0.00
T <sub>3</sub> C <sub>1</sub> (15 <sup>th</sup> July+IBA @ 1500 ppm)	38.10	65.56	11.10	7.85	1.42	22.20
T <sub>3</sub> C <sub>2</sub> (15 <sup>th</sup> July+IBA @ 3000 ppm)	32.60	84.44	18.20	11.90	1.61	44.40
T <sub>3</sub> C <sub>3</sub> (15 <sup>th</sup> July+IBA @ 4500 ppm)	28.40	92.20	24.80	14.20	1.86	70.00
T <sub>3</sub> C <sub>0</sub> (15 <sup>th</sup> July+IBA @ 0 ppm)	43.50	54.44	6.96	7.01	1.22	8.89
T <sub>4</sub> C <sub>1</sub> (30 <sup>th</sup> July+IBA @ 1500 ppm)	41.40	62.20	7.52	6.90	1.32	11.10
T <sub>4</sub> C <sub>2</sub> (30 <sup>th</sup> July+IBA @ 3000 ppm)	37.70	72.20	11.30	9.07	1.46	33.30
T <sub>4</sub> C <sub>3</sub> (30 <sup>th</sup> July+IBA @ 4500 ppm)	30.80	86.70	18.60	12.30	1.59	46.70
T <sub>4</sub> C <sub>0</sub> (30 <sup>th</sup> July+IBA @ 0 ppm)	48.40	46.67	4.48	4.01	1.16	3.33
Mean	40.07	63.06	11.72	8.51	1.41	26.60
SE(m)	0.64	1.84	0.48	0.47	0.015	1.85
SE(d)	0.90	2.61	0.68	0.66	0.021	2.61
C.D. at 5%	1.79	5.17	1.35	1.31	0.041	5.18
Significance	*	*	*	*	*	*

## CONCLUSION

As per the findings of this experiment, time of air layering and IBA concentrations had a positive significant effect on the rooting behaviour and success of rooting of air layers in Pant Prabhat guava. Air layering performed during 15<sup>th</sup> July gave best rooting performance and significant results in rooting characteristics. In respect to IBA concentrations, 4500 ppm showed best results for all the rooting parameters comparison to control. Therefore, on the basis of above conclusion, it is recommended that air layering time 15<sup>th</sup> July with 4500 ppm IBA is suitable for best rooting performance of air layers of Pant Prabhat guava under sub-tropical condition of Garhwal Himalaya.

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## EFFICACY OF INSECTICIDE AGAINST INSECT PEST OF SOYBEAN, *GLYCINE MAX (L.) MERRIL*

Nikki Bhardwaj<sup>1\*</sup>, S.B. Singh, Pavithra S.<sup>1</sup> and K.K. Singh<sup>2</sup>

<sup>1</sup> *Rajmata Vijayaraje Rajmata Vijayaraje Scindia Krishi Vishwavidyalaya Gwalior.(M.P.)*

<sup>2</sup> *Department of Agriculture, Career Point University, Kota, Rajasthan, India*

Email: [nikkibhardwaj7610@gmail.com](mailto:nikkibhardwaj7610@gmail.com)

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**Abstract:** Efficacy of Quinalphos 25 EC, Imidacloprid 17.8 SL, Trizophos 40 EC, Chlorpyrifos 20 EC, Alphamethrin 10 EC, Profenophos 50 EC and Dimethoate 30 EC was observed. The overall maximum reduction in girdle beetle infestation was noticed in Quinalphos 25 EC (82.15%) followed by Alphamethrin 10 EC (79.41%), and it was minimum in Chlorpyrifos 20 EC (75.88%). The blue beetle population reduction was noticed maximum in Trizophos 40 EC (87.81%) followed by Profenophos 50 EC (85.61%) and minimum in Alphamethrin 10 EC (81.41%). The maximum reduction in green semilooper population was recorded in Profenophos 50 EC (88.05%) followed by Imidacloprid 17.8 SL (87.98%), and minimum in Quinalphos 25 EC (84.57%). Tobacco caterpillar showed maximum population reduction in Imidacloprid 17.8 SL (90.24%) followed by Quinalphos 25 EC (89.42%), and it was minimum in Profenophos 50 EC (86.40%). The Highest grain yield (kg./ha.) was recorded in Imidacloprid 17.8 SL (1500) and it was minimum in Quinalphos 25 EC (850). The best cost benefit ratio was noted in Imidacloprid 17.8 SL (1:3.42) followed by Trizophos 40 EC (1:3.20), and lowest in Quinalphos 25 EC (1:1.94).

**Keywords:** Insecticide, Insect, Soybean, Control

### INTRODUCTION

Soybean (*Glycine max (L.) Merrill*) is known as the "Golden Bean" of the twentieth century. Though soybean is a legume crop, yet it is widely used as oilseed. It can be grown on a variety of soil and in a wide range of climate. Soybean is a *kharif* crop in India, sown in June-July and harvested in late September–October. Peak arrivals begin from October and November. It has emerged as an important commercial crop in many countries and international trade of soybean is spread globally. Nationally it occupies an area of 110.65 lakh ha and its production is 69.29 lakh MT. Madhya Pradesh ranks first in soybean production in India. Area and production of soybean in Madhya Pradesh are 56.12 lakh ha and 34.12 lakh MT, respectively (Sopa, 2015).

The major soybean producing states are Madhya Pradesh, Maharashtra, Rajasthan, Karnataka, Uttar Pradesh, Andhra Pradesh and Gujarat. It is a result of accumulating year in Soybean production, change in cropping practices, or global climate change, the distribution and impact of native and established pest is increasing in soybean. Soybean is affected by many species of insect pests (Aske *et al.*, 2007). The populations of foliar insect pests including sucking pests and pod feeders such as bean leaf beetle, stink bugs and other pests, are increasing in many regions and efforts are being made to manage them. A number of insecticides have been tested earlier and exhibited effectiveness, but losses due to insect pests are still not below the economic injury level. It has been noticed that in last few years traditional insecticides are not in use due their resistance but

after long interval their efficacy is again required to be tested. Chemical control strategies remain the main tool in the suppression of soybean defoliators. In the past, defoliators were controlled using broad spectrum insecticides such as Dimethoate 30 EC, Imidacloprid 17.8 SL, Trizophos 40 EC, Chlorpyrifos 20 EC, Alphamethrin 10 EC, Profenophos 50 EC, Quinalphos 25 EC,

The soybean defoliators mainly include tobacco caterpillar (*Sopdoptera litura* Fab.) and green semilooper (*Chrysodeixis acuta*). Immature stages (larva or caterpillar) of both tobacco caterpillar and green semilooper damages the crop at vegetative stage and in severe case, it completely defoliate the crop and dramatic yield loss. *S. litura* larvae even damages to soybean pods also (Sastawa *et al.*, 2004). The control of pest in crop cost high to the farmer. The study conducted in the year 2009, among soybean loopers, *Chrysodeixis acuta* and observed that these pests infested 1.7 million acres of soybeans and caused a 19 % total loss plus cost of control to producers (Musser, and Catchot, 2009). Chemical control strategies remain the main tool in the suppression of soybean insect pest. In the past, defoliators were controlled using broad spectrum insecticides such as organochlorins, organophosphates, synthetic pyrethroids and carbamates. Overuse and reliance on these insecticides led to many documented cases of resistance of virtually all classes of insecticides (Brewer *et al.*, 1990 and Wolfenbarger and Brewer, 1993). Today, insecticides applications are mainly limited to lepidopteran- specific compounds and newer chemistries of insecticides such as diamides. Presently the insecticides recommended for the

\*Corresponding Author

control of defoliators are methomyl (carbamate), indoxacarb (oxadiazine), spinosad (spinosyn) and flubendiamide (diamide). It is known fact that these both lepidopteron defoliators showed certain levels of behavioral resistance to different class of insecticides, hence successful control of this pest is some extent difficult. Keeping this in view, study were under taken to test the effectiveness of some newer group of molecules against these pest in soybean.

## MATERIAL AND METHOD

The trials were laid out during the year 2015-16 in a randomized block design having plot size of  $5 \times 2.7$  m<sup>2</sup> at, experimental farm. The cultivar RVS 2001-4 was sown on 27 June 2015 with all the recommended agronomical practices were followed by College of Agriculture, Indore except insect pest management. Different treatments comprising of seven insecticides, as per the details given in the Table 1 were applied with the help of manually operated hand knapsack sprayer. There were total of 8 treatments including untreated check replicated thrice.

Observations on larval population and percent pod damage were recorded procedure given by (Harish, 2008). Three randomly spots of one square meter row in each treatment leaving border rows. Larval count was made by shaking the plant gently over a white cloth placed between the rows. Average number of caterpillars found per square meter row was worked out for pre count 3,7 and 14 days after Spraying For percent pod damage, ten plants are randomly selected from each plot and total number of pods and damaged pods at the time of harvesting were recorded and mean was calculated. Percent pod damage was calculated by following formula.

$$\text{Percent pod damage} = \frac{\text{Number of damaged pods}}{\text{Total no. of pod}} \times 100$$

At the time of harvesting, yield from each plot was taken separately and converted into kg/ha and statistically analyzed. Data obtained were subjected to analysis of variance (ANOVA) after transformation of data through CPCS-I software and as per the procedure suggested by Gomez and Gomez (1984).

**Table 1.** Detail of different treatments

Treatment	Name of insecticides	Tread name	Recommended concentration (%)	Class	Mode of action	Dose g.a.i./ha
T1	Quinolphos 25EC	Ekalux AF	0.05	Organophosphate	Contact & Stomach	250
T2	Imidacloprid 17.8SL	Confidor	0.004	Neonicotinoid	Contact & Stomach	19.93
T3	Trizophos 40EC	Hostathion	0.06	Organophosphorus	Contact & Systemic	300
T4	Chlorpyriphos 20EC	Dursban	0.06	Organophosphate	Stomach action	300
T5	Alphamethrin 10EC	Guru	0.003	Synthetic Pyrethroid	Contact & Stomach	15
T6	Prophenophos 50EC	Curacron	0.05	Organophosphorus	Contact & Stomach	250
T7	Dimethoate 30EC	Rogar	0.06	Organophosphate	Systemic action	300
T8	Control	-	-	-	-	-

\* Figures in the parenthesis are angular transformed values.

## RESULT AND DISCUSSION

IT was observed that soybean crop was heavily attacked by soybean insect and defoliators during the season. Results of the present investigation, Efficacy of insecticide against insect pest of soybean, *Glycine Max (L.) Merril* are elucidated here. There were significant differences among the treatments applied for the soybean insects.

### Effect of treatments on girdle beetle infestation

The overall reduction in girdle beetle population after three applications of treatments over pre treatment population of first application to the last count of third spray was calculated. (Table 2) The result revealed that the maximum reduction in population was noticed in quinalphos 25 EC (82.15%) followed by

alphamethrin 10 EC (79.41%), trizophos 40EC (78.37%), imidacloprid 17.8 SL (78.61%), dimethoate 30 EC (77.41%) and profenphos 50 EC (77.18%) and it was minimum in chlorpyriphos 20EC (75.88%). Kalyan and Ameta (2016) studied two sprays given in the soybean, of which first spray was given against semilooper and girdle beetle at 35 days after germination (DAG) and second spray was given at 55 DAG against gram pod borer and tobacco caterpillar. It was followed by imidacloprid 200 SL, while the significant highest reduction in the larval population of semilooper and girdle beetle was recorded in case of Profenophos 50 EC at 3 and 5 (DAS). It was followed by triazophos 40 EC and dimethoate 30 EC against semilooper and girdle beetle, respectively. Similar trends were also observed at 7 DAS.

**Table 2.** Effect of different insecticide on soybean Girdle beetle (pooled data of 2015-16).

Treatment	Doses g.a.i. ha <sup>-1</sup>	Pre-treatment count	After 1 <sup>st</sup> spray			After 2 <sup>st</sup> spray			After 3 <sup>st</sup> spray		
			3 DAS	7 DAS	14 DAS	3 DAS	7 DAS	14 DAS	3 DAS	7 DAS	14 DAS
Quinolphos 25EC	250	10.86 (19.21)	6.22 (14.44)	6.33 (14.57)	6.43 (14.68)	4.64 (12.38)	4.79 (12.62)	4.86 (12.71)	2.68 (9.41)	2.73 (9.51)	2.82 (9.66)

Imidacloprid 17.8SL	19.93	9.731 (8.00)	5.86 (13.96)	6.05 (14.22)	6.14 (14.33)	3.73 (11.11)	3.87 (11.32)	3.97 (11.47)	2.13 (8.39)	2.23 (8.59)	2.31 (8.72)
Trizophos 40EC	300	10.84 (19.19)	6.29 (14.48)	6.38 (14.60)	6.44 (14.67)	4.50 (12.24)	4.76 (12.60)	4.83 (12.69)	2.85 (9.70)	2.91 (9.81)	3.00 (9.96)
Chlorpyriphos 20EC	300	11.57 (19.87)	7.46 (15.82)	7.51 (15.87)	7.58 (15.96)	4.74 (12.54)	4.98 (12.88)	5.05 (12.97)	3.15 (10.17)	3.23 (10.31)	3.35 (10.51)
Alphamethrin 10EC	15	10.64 (18.99)	6.88 (15.20)	6.95 (15.28)	7.05 (15.38)	5.63 (13.69)	5.77 (13.86)	5.86 (13.98)	3.89 (11.33)	3.98 (11.47)	4.05 (11.59)
Prophenophos 50EC	250	10.49 (18.87)	6.03 (14.21)	6.16 (14.36)	6.35 (14.60)	3.82 (11.25)	3.92 (11.39)	4.04 (11.55)	2.75 (9.52)	2.95 (9.84)	3.06 (10.04)
Dimethoate 30EC	300	11.27 (19.60)	7.85 (16.24)	7.97 (16.36)	8.05 (16.46)	4.47 (12.21)	4.56 (12.32)	4.68 (12.49)	2.77 (9.56)	2.94 (9.83)	3.27 (10.37)
Control	-	14.34 (22.24)	14.24 (22.17)	14.30 (22.21)	14.30 (22.21)	14.23 (22.16)	14.17 (22.10)	14.26 (22.18)	14.33 (22.24)	14.25 (22.17)	14.20 (22.13)
		<b>8.42</b>	<b>6.99</b>	<b>6.03</b>	<b>5.11</b>	<b>6.90</b>	<b>6.47</b>	<b>6.41</b>	<b>7.67</b>	<b>8.11</b>	<b>8.20</b>
		<b>NS</b>	<b>0.88</b>	<b>0.91</b>	<b>0.91</b>	<b>0.92</b>	<b>0.94</b>	<b>0.95</b>	<b>0.49</b>	<b>0.52</b>	<b>0.56</b>

\* Figures in the parenthesis are angular transformed values

**Effect of treatments on blue beetle population**

The overall reduction in blue beetle population after three applications of treatments over pre treatment population of first application to the last count of third spray was calculated.(Table 3) The result revealed that the maximum reduction in population was noticed in Trizophos 40EC (87.81%) followed by Profenophos 50 EC (85.61%), Dimethoate 30 EC (84.92%), Imidacloprid 17.8 SL (84.51%), Chlorpyriphos 20EC (83.93%), Quinalphos 25 EC

(83.86%), and T5-alphamethrin 10 EC (81.41%). Kothalkar et al. (2015) revealed that Emamectin benzoate 5 SG @ 0.002% + Trizophos 40 EC @ 0.06%, Emamectin benzoate 5 SG @ 0.002%, Fenvalrate 20 EC @ 0.01%, Trizophos 40 EC @ 0.06% and Flubendiamide 20 WG @ 0.01% + Trizophos 40 EC @ 0.06% were proved to be significantly effective in managing the major insect pests of soybean. The results of the workers are in agreement with present study.

**Table 3.** Effect of different insecticide on soybean Blue beetle (pooled data of 2015-16).

Treatment	Doses g.a.i. ha-1	Pre-treatment count	After 1 <sup>st</sup> spray			After 2 <sup>nd</sup> spray			After 3 <sup>rd</sup> spray		
			3DAS	7 DAS	14 DAS	3DAS	7 DAS	14 DAS	3 DAS	7 DAS	14 DAS
Quinolphos 25EC	250	5.02 (2.35)	2.90 (1.84)	2.97 (1.86)	3.03 (1.88)	1.97 (1.57)	2.10 (1.61)	2.17 (1.63)	0.74 (1.11)	0.76 (1.12)	0.81 (1.14)
Imidacloprid 17.8SL	19.93	4.07 (2.14)	2.60 (1.76)	2.70 (1.79)	2.77 (1.81)	1.43 (1.39)	1.50 (1.41)	1.57 (1.44)	0.53 (1.01)	0.56 (1.03)	0.63 (1.06)
Trizophos 40EC	300	5.25 (2.39)	3.01 (1.87)	3.09 (1.89)	3.19 (1.92)	1.47 (1.40)	1.54 (1.43)	1.61 (1.45)	0.55 (1.03)	0.59 (1.04)	0.64 (1.07)
Chlorpyriphos 20EC	300	4.73 (2.28)	2.87 (1.83)	2.93 (1.85)	3.07 (1.88)	1.73 (1.49)	1.78 (1.51)	1.85 (1.53)	0.67 (1.08)	0.71 (1.10)	0.76 (1.12)
Alphamethrin 10EC	15	5.11 (2.37)	2.97 (1.86)	3.07 (1.89)	3.17 (1.91)	1.87 (1.53)	1.93 (1.55)	2.03 (1.59)	0.84 (1.15)	0.88 (1.17)	0.95 (1.20)
Prophenophos 50EC	250	5.17 (2.37)	2.94 (1.85)	3.04 (1.87)	3.14 (1.90)	1.82 (1.52)	1.90 (1.55)	1.97 (1.57)	0.72 (1.10)	0.77 (1.13)	0.82 (1.15)
Dimethoate 30EC	300	5.24 (2.39)	2.98 (1.86)	3.08 (1.89)	3.15 (1.91)	1.67 (1.47)	1.73 (1.49)	1.83 (1.53)	0.69 (1.09)	0.73 (1.11)	0.79 (1.13)
Control	-	6.73 (2.69)	6.80 (2.70)	6.73 (2.69)	6.87 (2.71)	6.97 (2.73)	7.03 (2.74)	7.07 (2.75)	7.15 (2.75)	7.05 (2.75)	6.88 (2.71)
		<b>12.14</b>	<b>9.43</b>	<b>9.41</b>	<b>9.09</b>	<b>9.07</b>	<b>6.93</b>	<b>7.90</b>	<b>6.66</b>	<b>5.19</b>	<b>6.74</b>
		<b>NS</b>	<b>0.22</b>	<b>0.22</b>	<b>0.21</b>	<b>0.17</b>	<b>0.18</b>	<b>0.17</b>	<b>0.13</b>	<b>0.10</b>	<b>0.13</b>

\* Figures in the parenthesis are angular transformed values.

**Effect of treatments on green semilooper population**

The overall reduction in green semilooper population after three applications of treatments over pre treatment population of first application to the last count of third spray was calculated.(Table 4) The result revealed that the maximum reduction in population was recorded in Profenophos 50 EC (88.05%) followed by Imidacloprid 17.8 SL (87.98%), Chlorpyriphos 20EC (86.92%), Dimethoate 30 EC (86.90%), Trizophos 40EC (86.63%), Alphamethrin 10 EC (86.40%) and Quinalphos 25 EC (84.57%). Kalyan and Ameta (2016) studied two

sprays given in the soybean, of which first spray was given against semilooper and girdle beetle at 35 days after germination (DAG) and second spray was given at 55 DAG against gram pod borer and tobacco caterpillar. It was followed by Imidacloprid 200 SL, while the significant highest reduction in the larval population of semilooper and girdle beetle was recorded in case of Profenophos 50 EC at 3 and 5 (DAS). It was followed by Trizophos 40 EC and Dimethoate 30 EC against semilooper and girdle beetle, respectively. Similar trends were also observed at 7 DAS.

**Table 4.** Effect of different insecticide on soybean Green semilooper (pooled data of 2015-16).

Treatment	Doses g.a.i. ha-1	Pre- treatment count	After 1 <sup>st</sup> spray			After 2 <sup>st</sup> spray			After 3 <sup>st</sup> spray		
			3 DAS	7 DAS	14 DAS	3 DAS	7 DAS	14 DAS	3 DAS	7 DAS	14 DAS
Quinolphos 25EC	250	3.50 (2.00)	1.77 (1.50)	1.87 (1.54)	1.93 (1.56)	0.82 (1.14)	0.85 (1.16)	0.88 (1.18)	0.46 (0.98)	0.51 (1.00)	0.54 (1.02)
Imidacloprid 17.8L	19.93	3.30 (1.94)	1.47 (1.40)	1.53 (1.42)	1.57 (1.44)	0.59 (1.04)	0.64 (1.07)	0.67 (1.08)	0.35 (0.92)	0.37 (0.93)	0.40 (0.95)
Trizophos 40EC	300	3.37 (1.96)	1.50 (1.41)	1.57 (1.44)	1.63 (1.46)	0.62 (1.06)	0.66 (1.08)	0.69 (1.09)	0.38 (0.94)	0.42 (0.96)	0.45 (0.97)
Chlorpyrifos 20EC	300	3.90 (2.10)	1.33 (1.35)	1.40 (1.38)	1.47 (1.40)	0.83 (1.15)	0.87 (1.17)	0.90 (1.18)	0.42 (0.96)	0.48 (0.99)	0.51 (1.00)
Alphamethrin 10EC	15	3.70 (2.05)	1.53 (1.42)	1.60 (1.45)	1.67 (1.47)	0.78 (1.13)	0.82 (1.15)	0.86 (1.16)	0.44 (0.97)	0.48 (0.99)	0.50 (1.00)
Prophenophos 50EC	250	4.10 (2.14)	1.63 (1.46)	1.70 (1.48)	1.77 (1.51)	0.72 (1.10)	0.76 (1.12)	0.80 (1.14)	0.41 (0.95)	0.45 (0.98)	0.49 (0.99)
Dimethoate 30EC	300	3.97 (2.11)	1.60 (1.45)	1.67 (1.47)	1.73 (1.49)	0.86 (1.16)	0.90 (1.18)	0.96 (1.21)	0.43 (0.97)	0.46 (0.98)	0.52 (1.01)
Control	-	4.47 (2.23)	4.54 (2.25)	4.61 (2.26)	4.68 (2.28)	4.74 (2.29)	4.80 (2.30)	4.73 (2.29)	4.78 (2.30)	4.72 (2.28)	4.68 (2.28)
		<b>8.35</b>	<b>7.60</b>	<b>5.47</b>	<b>4.25</b>	<b>6.83</b>	<b>4.69</b>	<b>4.21</b>	<b>1.41</b>	<b>3.49</b>	<b>2.71</b>
		NS	<b>0.16</b>	<b>0.11</b>	<b>0.09</b>	<b>0.13</b>	<b>0.09</b>	<b>0.08</b>	<b>0.02</b>	<b>0.08</b>	<b>0.05</b>

\* Figures in the parenthesis are angular transformed values.

#### Effect of treatments on tobacco caterpillar population

The result revealed that the maximum reduction in population was noticed in Imidacloprid 17.8 SL (90.24%) followed by Quinalphos 25 EC (89.42%), Chlorpyrifos 20EC (88.99%), Trizophos 40EC (88.25%), Dimethoate 30 EC (88.17%) and Alphamethrin 10 EC (88.12%) and it was minimum in profenphos 50 EC (86.40%). Data of the efficacy of insecticide against *S. litura* is presented in Table 5. Chari et al., (1999) indicated that neem azal F I and II at 50 ppm concentration, NSKS 2% and Chlorpyrifos 0.05% gave significant protection to

tobacco seedling from the *Spodoptera* damage followed by neem azal F II (25 ppm) and neem azal I (30 ppm). Yadav et al., (2001) reported that *Spodoptera litura* (Fab.) treatment Chlorpyrifos 50 EC + Cypermethrin 5 EC was found to be the most effective in reducing the population of tobacco caterpillar; larvae. Treatments viz. Chlorpyrifos 50 EC + Cypermethrin 5 EC and Profenophos + Cpermethrin 44 EC were effective in keeping the larval population below 2 larvae per metre. Purwar and Yadav (2003) reported that triazophos was found effective against *Spodoptera litura* larvae on two cultivars of soybean crop i.e., PK-1029 and PK-416.

**Table 5.** Effect of different insecticide on soybean Tobacco caterpillar (pooled data of 2015-16).

Mean number of grub per sq m row

Treatment	Doses g.a.i. ha-1	Pre- treatment count	After 1 <sup>st</sup> spray			After 2 <sup>st</sup> spray			After 3 <sup>st</sup> spray		
			3 DAS	7 DAS	14 DAS	3 DAS	7 DAS	14 DAS	3 DAS	7 DAS	14 DAS
Quinolphos 25EC	250	4.63 (2.26)	2.20 (1.64)	2.27 (1.66)	2.33 (1.68)	1.09 (1.26)	1.10 (1.26)	1.13 (1.28)	0.44 (0.97)	0.46 (0.98)	0.49 (0.99)
Imidacloprid 17.8SL	19.93	4.20 (2.16)	2.07 (1.60)	2.11 (1.61)	2.14 (1.62)	1.02 (1.23)	1.06 (1.25)	1.09 (1.26)	0.36 (0.93)	0.38 (0.94)	0.41 (0.95)
Trizophos 40EC	300	4.00 (2.12)	2.00 (1.58)	2.09 (1.61)	2.15 (1.63)	1.01 (1.23)	1.04 (1.24)	1.08 (1.25)	0.42 (0.96)	0.44 (0.97)	0.47 (0.98)
Chlorpyrifos 20EC	300	4.51 (2.230)	2.27 (1.66)	2.34 (1.68)	2.37 (1.69)	1.03 (1.24)	1.11 (1.26)	1.15 (1.28)	0.43 (0.96)	0.46 (0.98)	0.50 (1.00)
Alphamethrin 10EC	15	4.63 (2.26)	2.30 (1.67)	2.37 (1.69)	2.44 (1.71)	1.05 (1.24)	1.09 (1.26)	1.12 (1.27)	0.49 (0.99)	0.52 (1.01)	0.55 (1.02)
Prophenophos 50EC	250	3.97 (2.11)	2.34 (1.68)	2.39 (1.70)	2.48 (1.73)	1.04 (1.24)	1.07 (1.25)	1.10 (1.26)	0.47 (0.98)	0.49 (0.99)	0.54 (1.02)
Dimethoate 30EC	300	4.31 (2.19)	2.17 (1.63)	2.22 (1.65)	2.28 (1.67)	1.06 (1.25)	1.12 (1.27)	1.19 (1.30)	0.45 (0.97)	0.48 (0.99)	0.51 (1.00)
Control	-	4.42 (2.22)	4.49 (2.23)	4.55 (2.25)	4.61 (2.26)	4.68 (2.28)	4.58 (2.25)	4.51 (2.24)	4.44 (2.22)	4.38 (2.21)	4.31 (2.19)
CV %		<b>7.46</b>	<b>3.90</b>	<b>4.15</b>	<b>4.25</b>	<b>4.53</b>	<b>6.31</b>	<b>7.69</b>	<b>6.81</b>	<b>6.59</b>	<b>5.15</b>
CD at 5 %		NS	<b>0.09</b>	<b>0.09</b>	<b>0.09</b>	<b>0.09</b>	<b>0.12</b>	<b>0.15</b>	<b>0.12</b>	<b>0.12</b>	<b>0.09</b>

\* Figures in the parenthesis are angular transformed values

**Cost benefit ratio****Grain yield (kg/ha)**

Data pertaining to yield and economics were presented in table no. 6. The highest grain yield (kg./ha.) was recorded in imidacloprid 17.8 SL (1500) and differed significantly with all the treatments. The next best treatment was trizophos 40 EC (1400) which also showed significant difference with remaining treatments. The third best treatment was observed as chlorpyrifos 20 EC (950) and found at par with followed by alphamethrin 10 EC (920), dimethoate 30 EC (910), profenophos 50 EC (870) and quinolphos 25 EC (850). Gupta (1998) reported the maximum yield (23.17 q/ha) with Profenophos closely followed by Ethion and Trizophos (23.0 q/ha each) as against conventional insecticide and untreated control (18.33 q/ha). Khandwe and Waghmare (2003) reported that two sprays given of chlorpyrifos at 40 and 45 DAS gave the highest yield (18.24 q/ha) and (Rs. 412/ha) of soybean.

**Cost benefit ratio**

The best cost benefit ratio was noted in imidacloprid 17.8 SL (1:3.15) followed by trizophos 40 EC (1:2.84), alphamethrin 10 EC (1:1.94), chlorpyrifos 20 EC (1:1.91) profenophos 50 EC (1:1.77), quinolphos 25 EC (1:1.75) and dimethoate 30 EC (1:1.74). In a study carried out by Kalyan and Ameta 2016 & recorded cost benefit ratio. Insecticidal spray schedule was comprising of first spray of triazophos 40 EC @ 1.25 l/ha at 35 DAG followed by second spray of flubendiamide 480 SC @ 100 ml/ha at 55 DAG provided the highest mean seed yield of 1925 kg/ha. The maximum net profit of Rs. 15, 008/ha was obtained in case of spray schedule comprising first spray of triazophos 40 EC @ 1.25 l/ha at 35 DAG followed by second spray of flubendiamide 480 SC @ 100 g/ha at 55 DAG with the maximum cost: benefit ratio of 1: 8.52. The minimum net profit of Rs. 3, 698/ha was obtained in first spray of monocrotophos 36 SL followed by second spray of triazophos 40 EC with the minimum cost: benefit ratio of 1: 3.32.

**Table 6.** Effect of different insecticide on soybean yield and cost economics (pooled data of 2015-16).

Treatments	Cost of cultivation (Rs/ha)	Quantity of insecticide used for 3 sprays (ml or gm/ha)	Cost of Insecticides (Rs ha <sup>-1</sup> )	Labour cost 2 labourers per spray per ha	Total cost (Rs)	Yield kg/ha	Gross income (Rs)	Net income (Rs)	Cost benefit ratio
T1- Quinolphos 25 EC	17500	3000	675	1200	19375	850	34000	16500	1:1.75
T2-Imidacloprid 17.8 SL	17500	336	344	1200	19044	1500	60000	40956	1:3.15
T3 – Trizophos 40 EC	17500	2250	1008	1200	19708	1400	56000	36292	1:2.84
T4 – Chlorpyrifos 20 EC	17500	4500	1126	1200	19826	950	38000	18174	1:1.91
T5- Alphamethrin 10 EC	17500	450	202	1200	18902	920	36800	17898	1:1.94
T6 –Profenophos 50 EC	17500	1500	862	1200	19562	870	34800	15238	1:1.77
T7 – Dimethoate 30 EC	17500	3000	2174	1200	20874	910	36400	15526	1:1.74
T8 – Untreated check	17500	----		1200	18700	540	21600	2900	--
<b>CD at 5 %</b>	-	-	-	-	-	<b>39.96</b>	-	-	-

Cost of cultivation (Rs/ha) 17500/-

**CONCLUSION**

1. The overall reduction in girdle beetle population after three applications of treatments over pre treatment population of first application to the last count of third spray was calculated. The result revealed that the maximum reduction in population was noticed in quinalphos 25 EC (82.15%) followed by alphamethrin 10 EC (79.41%), trizophos 40 EC (78.37%), imidacloprid 17.8 SL (78.61%), dimethoate 30 EC (77.41%) and profenophos 50 EC (77.18%) and it was minimum in chlorpyrifos 20 EC (75.88%).
2. The overall reduction in blue beetle population after three applications of treatments over pre treatment population of first application to the last count of third spray was calculated. The result revealed that the maximum reduction in population was noticed in trizophos 40 EC (87.81%) followed by profenophos 50 EC (85.61%), dimethoate 30 EC (84.92%), imidacloprid 17.8 SL (84.51%),

chlorpyrifos 20 EC (83.93%), quinalphos 25 EC (83.86%), and alphamethrin 10 EC (81.41%).

3. The overall reduction in green semilooper population after three applications of treatments over pre treatment population of first application to the last count of third spray was calculated. The result revealed that the maximum reduction in population was recorded in profenophos 50 EC (88.05%) followed by imidacloprid 17.8 SL (87.98%), chlorpyrifos 20 EC (86.92%), dimethoate 30 EC (86.90%), trizophos 40 EC (86.63%), alphamethrin 10 EC (86.40%) and quinalphos 25 EC (84.57%).

4. The result revealed that the maximum reduction in tobacco caterpillar population was noticed in imidacloprid 17.8 SL (90.24%) followed by quinalphos 25 EC (89.42%), chlorpyrifos 20 EC (88.99%), trizophos 40 EC (88.25%), dimethoate 30 EC (88.17%) and alphamethrin 10 EC (88.12%) and it was minimum in profenophos 50 EC (86.40%).

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## YIELD IRRIGATION PRODUCTION EFFICIENCY AND ECONOMIC RETURN OF GREEN PEA UNDER VARIABLE IRRIGATION AND FERTIGATION

Arvind Kumar<sup>1</sup>, Avadhesh Kumar Koshal<sup>2\*</sup>, M. Imtiyaz<sup>3</sup> and S.K. Srivastava<sup>4</sup>

<sup>1,3,4</sup>Department of soil water land engineering and management, Vauge school of agricultural and technology, SHIATS, Allahabad U.P. 211007

<sup>2</sup>Faculty of Sciences, Motherhood University, Roorkee, Haridwar, Uttarakhand 247661  
Email: [akkoshal@hotmail.com](mailto:akkoshal@hotmail.com)

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**Abstract:** Field study was carried out at the irrigation Research Farm of Sam Higginbottom Institute of Agriculture and Sciences (Deemed to be University) Allahabad, U. P., India, During winter crop growing season of December 2011 to March 2012, on clay loam soil in order to evaluate the yield of green pea under different irrigation scheduling with fertigation under semi arid climate. The crop was subjected to variable irrigated level (IW/CPE ratio of 50, 75, 100, 125, 150) and fertigation level (100, 200, 300). The crop was irrigated when daily mean of USWB class. A pan evaporation reached to predetermined value of 16.3 mm. irrigated by drip irrigation method with 4l/h non compensated on line dripper's Irrigation at 125% of pan evaporation replenishment and fertigation level 300kg/ha resulted in higher green pea yield, whereas irrigation production efficiency was higher with irrigation at 50% of pan evaporation replenishment with fertigation level 300kg/ha. The irrigation at 125% with fertigation level of 300kg/ha, of Pan –evaporation replenishment resulted in higher gross return, net return and benefit cost ratio. Seasonal water applied irrigation schedules and bulb yield, gross return, net return and benefit cost ratio exhibited strong quadratic relationship which can use for optimizing green pea production in this region.

**Keywords:** Drip irrigation, Irrigation scheduling, Fertigation, Economic analysis marketable yield, Pea

### INTRODUCTION

Water resource is the major constraint for crop diversification and production in India. India has the second largest net irrigated area in the world, after china. The irrigation efficiency under canal irrigation is not more than 40% and for ground water schemes, it is 69%. The net irrigated area in the country is 53.5Mha, which is about 38% of the total sown area. Although considerable area has been brought under irrigation since independence; There are several causes that limit water availability for agriculture an increasing demand by the civil users a greater requirement by the industrial sector; climate changes that cause a rise in the air temperature and an irregular distribution of rainfall, producing more intense precipitations and runoffs and limiting water infiltration in the soil as well as the refill of the aquifers; a scarce maintenance of the water distribution network. Therefore, it is necessary to develop irrigation management strategies in order to use scarce water resources efficiently and effectively for vegetable production.

Scheduling the irrigation is all about deciding by amount and frequency of irrigation. The amount of water to be applied during each irrigation was determined by IW/Epan, the ratio between a fixed amount of irrigation water (IW) and cumulative open evaporation (Epan) minus rainfall. Therefore, it is important to develop a proper and effective scheduling of irrigation under prevailing climatic condition to obtained maximum benefit from the available limited water resources. Numerous studies

have been carried out in the past on the development and evaluation of irrigation scheduling techniques under a wide range of irrigation system and management, soil, crop and climatic conditions (Stewart, 1975). Drip irrigation has gained widespread acceptance as an efficient and economically viable method due to its highly localized application of water and nutrient to crop. Pea (*Pisum sativum* L.) is an important frost hardy cool-season leguminous vegetables crop that is widely cultivated throughout the world.

### MATERIAL AND METHOD

The experimental was conducted at the Irrigation Research Farm of the Department of Soil water land Engineering and management in the Vauge school of Agriculture Engineering and Technology SHIATS, Allahabad (UP). The irrigation research station is situated at an elevation of 98 meter above sea level at 25.87°N latitude and 81.15°E longitude and has a tropical to sub tropical climate with extremes of summer and winter. During the winter months average temperature range from 5°C to 1°C while in the summer season the temperature varies from 3°C to 45°C. The soil of the experimental field was fertile clay loam. (35.5% sand, 25.8% silt and 38.6% clay) with average bulk density of 1.31 gm/cm. The moisture content at field capacity (-1/3 bar) and wilting point (-1/5 bar) was 19.5% and 9.1% on an oven dry weight loss basis respectively. The experiment was laid out in a three factor complete randomized block design. It comprises of 15

\*Corresponding Author

treatments with five irrigation levels and three fertigation levels. The area of each experimental plot was 5.4sqm (3x1.8m). Total number of plot was 45. A buffer zone spacing of 1.0 m was provided between the plots. Each plot was irrigated and fertigated independently, improved variety of pea was sown directly on 14<sup>th</sup> December 20012 at a spacing of row to row 30 cm and plant to plant 10 cm. Before planting, experimental field of pea was well irrigated. After 15 days of sowing, irrigation treatments were started. Drip irrigation crop receive first nutrient dose as booster dose after 15 days of sowing, whole recommended dose of P and K and half of N fertilizer was applied in before sowing. In case of fertigation, fertilizer was applied in six doses at regular interval of 10 days.

The irrigation treatment comprised of five level of pan evaporation replenishment (50, 75, 100, 125, and 150%). And three fertigation level (100, 200, 300 kg/ha).Crop were irrigated when sum of the daily USWB class-A open pan evaporation data for a period of 6 years (2005-2010) were collected from meteorological station, SHIATS. The crop was irrigated when the sum of daily mean (6years) of pan evaporation reached to a predetermined value of 16.3 mm (rooting depth in m  $\times$  plant available water soil

moisture in mm /m  $\times$  permissible soil moisture depletion in fraction). The crop was irrigated by drip irrigation method. The irrigation water was pumped directly from the tank into the main line, sub-main and then into the plot through the laterals.PVC pipe of 50 mm diameter and low density polyethylene pipes (LDPE) of 12 mm diameter were use for main and lateral lines respectively. A control valve and water meter was connected to the sub- main line in order to monitor the amount of water application in respective treatment. Screen filter was installed on main line to minimize clogging of dipper and a control valve was connected to each experimental plot in order to deliver the desired amount of water. In drip irrigation system, lateral lines were laid to crop row system. The discharge of non pressure compensated online dripper was 4 l/ hr. The standard cultural practices were performed during the crop growing season. Green pea was harvested from 10<sup>th</sup> - 24<sup>th</sup> March 2012, respectively.

In order to assess the economic viability of different system under variable irrigation, both fixed and operating costs were included. The total cost of production, gross return and net return under different irrigation level were estimated under following assumption

Salvage value of the components	=	0
Useful life of tube-well, pump motor & pump house	=	25 years
Useful life of drip irrigation system	=	8 years
Useful life of weeding and spraying equipments	=	7 years
Interest rate	=	11.5%
Repair and maintenance	=	7.5%
Number of crops/year	=	2

The fixed costs which include tube well, pump, motor pump house and irrigation systems, PVC pipe for main and sub main and LDPE pipes for lateral, fertilizer tank, pressure gauges, water meter, drippers, spraying and weeding equipments for different methods and schedules which were calculated by the approach (James and Lee, 1971):

$$CRF = \frac{i(1+i)^n}{(1+i)^n - 1}$$

Where,

CRF	=	Capital recovery factor
I	=	Interest rate (fraction)
N	=	Useful life of the components (years)
Annual Fixed cost/ha	=	CRF X fixed cost/ha
Annual cost /ha/season	=	$\frac{\text{Annual fixed cost/ha}}{2}$

The operating cost which includes labor (system installation, fertilizer, chemical application and harvesting etc.);land preparation, seeds, fertilizer, chemicals (insecticides and pesticides) and water pumping (electricity) and repair and maintenance (tube well pump, motor, pump house, irrigation systems and pipe conveyance system etc.) was estimated. The gross return for different irrigation methods and schedules was calculated taking into consideration of marketable yield and wholesome price of green pea. Subsequently, the net return for green pea was calculated considering total cost of production (fixed and operating) and grosses return.

Total cost of production (Rs)	=	Fixed cost (Rs) + Operating cost (Rs)
Gross return (Rs/ha)	=	Marketable yield (t/ha) x wholesale price of green pea (Rs/t)
Net return (Rs/ha)	=	Gross return (Rs/ha) – Total cost of production (Rs/ha)
Benefit cost ratio (B/C)	=	Gross return (Rs/ha) / Total cost of production (Rs/ha)

## RESULT AND DISCUSSION

### i. Marketable Yield and irrigation production efficiency

The marketable pod yield and irrigation production efficiency of green pea as influenced by different irrigation and fertigation levels are presented in Table 1. The irrigation levels and fertigation

significantly influenced the marketable pod yield of green pea. The pod yield for different irrigation level ranged from 6.31 to 11.27 t/ha. The highest mean pod yield (11.27 t/ha) was recorded when irrigation during the crop growing season was applied at IW/CPE ratio of 125% ( $I_4$ ) with fertigation level  $F_1$  (200kg/ha). A further increase in irrigation level resulting from 150% of pan evaporation replenishment reduced the marketable pod yield (10.21 t/ha) due to poor aeration caused by excessive soil moisture. The fertigation level has significantly effect on marketable pod yield of green pea Table 1. Significantly higher marketable pod yield (10.26 t/ha) was recorded with fertigation levels  $F_3$  (300 kg/ha) of nutrient was applied, compared with 200 kg/ha ( $F_2$ ) and 100 kg/ha ( $F_1$ ) fertigation levels. The irrigation production efficiency of green pea was significantly influenced by irrigation levels and fertigation levels (Table1). The irrigation production efficiency for different irrigation levels range for 2.83 to 5.25 kg/m<sup>3</sup> the highest irrigation production

efficiency (5.25 kg /m<sup>3</sup>) was observed when irrigation was applied at 50% at pan evaporation replenishment because reduction in marketable yield was less as compared with seasonal water applied. Irrigation at 150% of pan evaporation replenishment resulted is significantly minimum irrigation production efficiency (2.83 kg/m<sup>3</sup>) because it increased seasonal water applied considerably but decreased the marketable pod yield (Table1). The overall results clearly revealed that both irrigation levels and fertigation levels in green pea influenced the marketable pod yield and irrigation production efficiency. The highest marketable pod yield was recorded at 125% of pan evaporation replenishment gave the higher irrigation production when fertigation walls provide at  $F_3$  300kg/ha levels. Imtiyaz et al. (2000a); reported the higher yield and irrigation production efficiency of vegetable crop at 80% of pan evaporation replenishment under the agro-climatic condition of northwestern Botswana.

**Table 1.** Effect of irrigation schedules and fertigation on marketable yield and irrigation production efficiency of green pea.

Treatment	Mean marketable green pod yield (t/ha)	Mean irrigation production efficiency (kg/m <sup>3</sup> )
Irrigation level		
50	6.31	5.25
75	7.29	4.05
100	9.35	3.89
125	11.27	3.75
150	10.21	2.83
LSD-0.717		
Fertigation level		
$F_1$	7.22	3.01
$F_2$	9.17	4.09
$F_3$	10.26	4.57
LSD-0.414		

## 2. Water applied and marketable pod yield

The relationship between seasonal water applied and marketable pod yield of green pea for three fertigation levels are presented in Fig. 4.1. The seasonal water applied ranged from 120 to 360mm, where as marketable pod yield for  $F_1$  (100kg/ha),  $F_2$  (200kg/ha) and  $F_3$  (300kg/ha) fertigation levels ranged from 5.00 to 9.10 t/ha, 6.62 to 11.65 t/ha and 7.33 to 13.06. t/ha respectively. The seasonal water and fertigation applied and marketable pod yield at green pea for 100kg ( $R^2= 0.9291$ ), 200kg ( $R^2= 0.8871$ ) and 300kg ( $R^2= 0.8893$ ) fertigation levels exhibited strong quadratic relationship. Green pea attained a maximum marketable pod yield at the seasonal water applied of 240 to 300 mm for  $F_1$  (100kg/ha),  $F_2$  (200kg/ha) and  $F_3$  (300kg/ha) fertigation levels respectively and their after it tended to decline. The relationship between pan evaporation replenishment are marketable pod yield at green pea 100kg/ha, 200kg/ha and 300kg/ha fertigation levels are presented in Fig. (4.2). The marketable pod yield

at green pea ranged from 5.00 to 9.10, 6.62 to 11.65 for and 7.33 to 13.06 t/ha for 100kg/ha, 200kg/ha and 300kg/ha fertigation levels respectively. The marketable yield and pan evaporation replenishment for 100kg/ha ( $R^2= 0.9291$ ), 200kg/ha ( $R^2= 0.8871$ ) and 300kg/ha ( $R^2= 0.8893$ ) fertigation levels exhibited strong quadratic relationship. Marketable pod yield at green pea increased with increase in pan evaporation replenishment approximately upto 125% for  $F_1$  (100kg/ha),  $F_2$  (200kg/ha) and  $F_3$  (300kg/ha) fertigation levels and their after it tended to decline. In spite of some variation the overall result show quadratic relationship between market able yield and seasonal water applied / pan evaporation replenishment under variable fertigation level. Imtiyaz et al.,(2000a) reported the quadratic relationships between marketable yield and seasonal water applied for cabbage, carrot, onion, tomato, broccoli spinach, green pepper, hot pepper, okra, egg plant and green maelies under both sprinkler and drip irrigation system. Many researchers have reported a

quadratic relationship between seasonal irrigation and yield of vegetable and field crop under a wide range of irrigation system and regimes, soil and climate (Tiwari and reddy, 1997; Zhang and Oweis, 1999).

### ii Economic return

The total cost of production, gross return, net return and benefit cost ratio of green pea as influenced by variable irrigation and fertigation level are presented in Table 2. The total cost of production increased slightly with an increase in irrigation and fertigation levels due to increase in water development with increase in amount of fertilizer applied. The highest gross return was obtained the irrigation levels with fertigation level  $I_4F_3$ , due to significantly higher marketable yield as compared with irrigation and fertigation levels. The net return increase significantly with increase in irrigation levels with increase in amount of fertilizer applied. The benefit cost ratio also increased considerably with increase in irrigation and fertigation levels. The highest benefit cost ratio was recorded when irrigation was applied at IW/ CPE ratio of 1.25  $I_4$  with fertigation level  $F_1$  (300kg/ha). Imtiyaz et al., (2000a) reported the similar results for cabbage, broccoli, onion, rape, tomato, carrot, spinach and green melies under drip and sprinkler irrigation.

### iii Relationship between Economic Return and Irrigation levels under fertigation levels

The Relationship between the seasonal water applied and gross return of green pea under variable fertigation levels are presented in (Fig. 4.3). Gross return ranged from 200000.00 to 330000.00 Rs/ha, 264800.00 to 424800.00 Rs/ha and 293200.00 to 471200.00 Rs/ha for 100kg/ha fertigation levels, 200kg/ha and 300kg/ha fertigation levels respectively. The gross return and seasonal water applied for 100kg/ha ( $R^2 = 0.9278$ ), 200kg/ha ( $R^2 = 0.8865$ ), and 300kg/ha ( $R^2 = 0.8844$ ) fertigation applied showed strong quadratic relationship. The

gross return of green pea increased with increase in seasonal water application upto 300 mm and thereafter it tended to decline. The green pea attained the maximum gross return at an IW/CPE ratio of 125%  $I_4$  with fertigation level  $F_3$  (300kg/ha) and thereafter the gross return tended to decline (Fig 4.4)

The Relationship between the net return and seasonal water applied of green pea under variable fertigation levels are presented in Fig. (4.5) The seasonal water applied and net return for 100kg/ha 200kg/ha and 300kg/ha fertigation levels exhibited strong quadratic relationship ( $R^2 = 0.9255$ ,  $R^2 = 0.886$ ,  $R^3 = 0.8867$ ). The green pea attained the maximum net return at 300 mm seasonal water applied application for all the three fertigation levels. The green pea attained the maximum net return at an IW/CPE ratio of 125%  $I_4$  with fertigation level  $F_3$  (300kg/ha) and thereafter the net return tended to decline (Fig 4.6)

The Relationship between the seasonal water applied and benefit cost ratio of green pea under variable fertigation levels are presented in Fig (4.7). The seasonal water applied and benefit cost ratio for 100kg/ha 200kg/ha and 300kg/ha fertigation levels exhibited strong quadratic relationship ( $R^2 = 0.9264$ ,  $R^2 = 0.8779$ ,  $R^2 = 0.87$ ). The green pea attained the maximum benefit cost ratio at 300 mm seasonal water applied application for all the three fertigation levels and thereafter benefit cost ratio tended to decline (Fig. 4.7). Similar trend were observed for irrigation level. The green pea attained the maximum benefit cost ratio at an IW/CPE ratio of 125%  $I_4$  with fertigation level  $F_3$  (300kg/ha) and thereafter the benefit cost ratio tended to decline (4.8). In spite of some variation, the overall result showed the strong quadratic relationship between seasonal water applied/ irrigation levels and gross return, net return and benefit cost ratio under variable fertigation levels which in turn can be use for optimizing the economic return of green pea with limited water resource condition. Similar results were reported by imtiyaz *et al.* (2000e, and 2004a) for different vegetable crop under varying soil, crop and climatic condition.

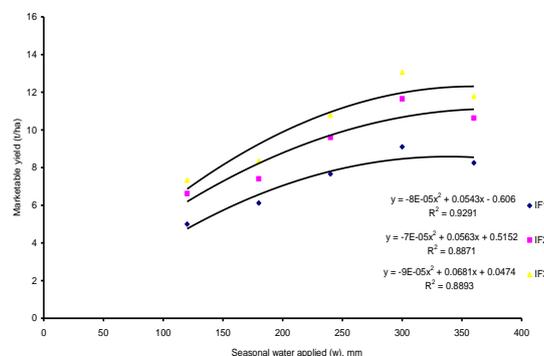
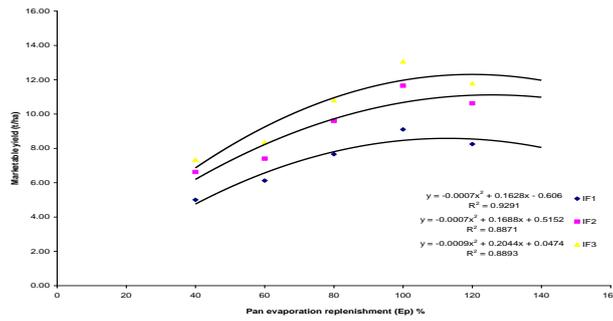
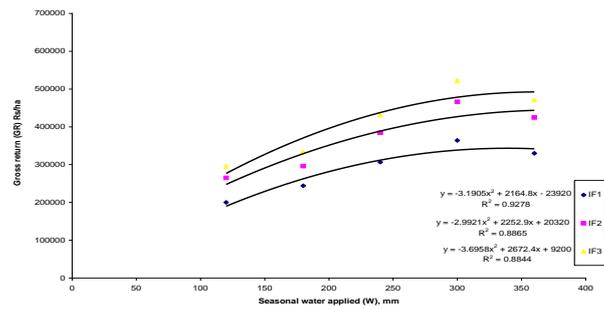


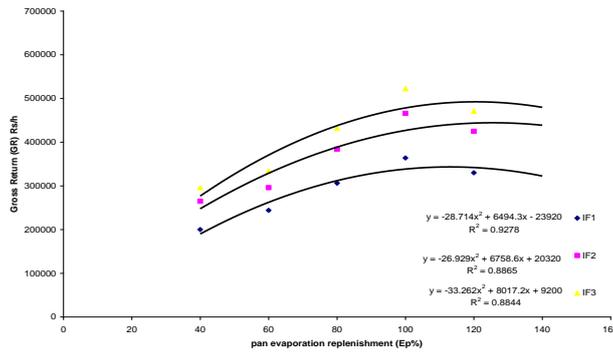
Fig. 4.1. Relationship between seasonal water applied and marketable yield of green pea for difference fertigation levels



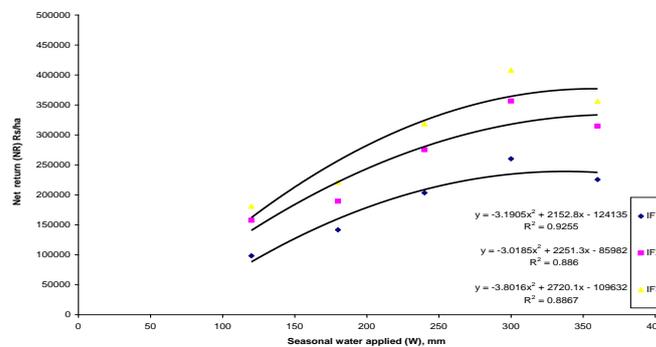
**Fig. 4.2** Relationship between pan evaporation replenishment and marketable yield of green pea for difference fertigation levels



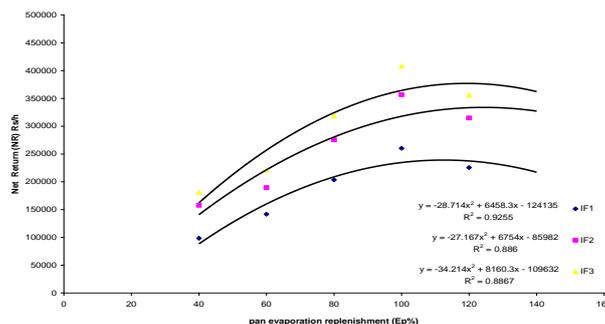
**Fig. 4.3.** Relationship between seasonal water applied and gross return of green pea for different fertigation levels



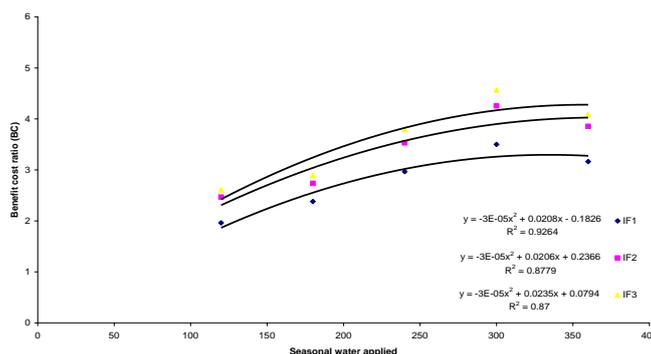
**Fig. 4.4** Relationship between pan evaporation replenishment and gross return green pea for different fertigation levels



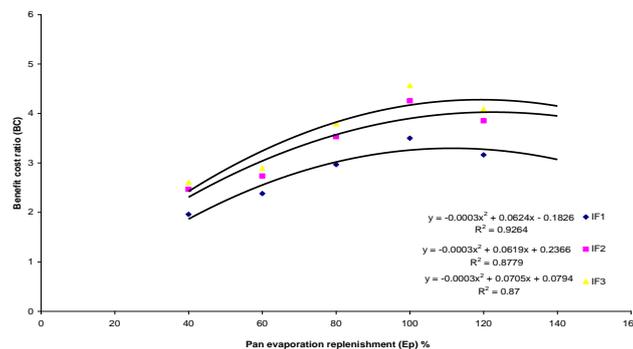
**Fig.4.5** Relationship between seasonal water applied and net return of green pea for different fertigation levels



**Fig.4.6.** Relationship between pan evaporation replenishment and net return of green pea for different fertigation levels



**Fig. 4.7.** Relationship between seasonal water applied and benefit cost ratio of green pea for different fertigation levels



**Fig. 4.8** Relationship between pan evaporation replenishment and benefit cost ratio of green pea for different fertigation levels

## CONCLUSION

Irrigation at 125 % of pan evaporation replenishment resulted in significantly higher green pod yield whereas irrigation production was higher with irrigation at 50% of pan evaporation replenishment. The fertigation levels of 300kg gave the higher marketable yield and irrigation production efficiency as compared with 100kg and 200kg fertigation levels. Irrigation at 125% of pan evaporation replenishment resulted in higher gross return, net return and benefit cost ratio. The fertigation level of F<sub>3</sub> 300kg gave higher net return as compared to 100kg and 200kg fertigation levels. The seasonal water applied / irrigation schedule and yield of green pea showed strong quadratic relationship for fertigation levels of 100, 200 and 300 kg fertigation levels exhibited a strong quadratic relationship. The seasonal water

applied / irrigation schedule and yield, gross return net return and benefit cost ratio under variable irrigation and fertigation levels exhibited a strong quadratic relationship which in turn can be under for allocation limited water resources for maximum return.

Finally the overall result clearly revealed that in order to obtain optimum yield and economic return, green pea should be irrigation at 125% of pan evaporation replenishment either with 100, 200 and 300kg/ha fertigation levels.

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## EFFECT OF POULTRY MANURE AND PSB CULTURE IN CONJUNCTION WITH DIFFERENT LEVELS OF PHOSPHORUS ON GROWTH AND YIELD OF BLACK GRAM (*VIGNA MUNGO L.*)

Raisen Pal\* and N. Swaroop

Department of Soil Science & Agricultural Chemistry, Sam Higginbottom University of Agriculture, Technology and Sciences- 211007 Allahabad, U.P.

Email: [raisenpalpreksha@gmail.com](mailto:raisenpalpreksha@gmail.com)

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**Abstract:** The experiment was conducted during *kharif* (July-Oct.) season 2016 on crop research farm of Department of Soil Science and Agricultural Chemistry, Naini Agricultural Institute, Allahabad. By order to evaluate the effect of different treatment of poultry manure and PSB culture with levels of phosphorus. The growth and yield parameters viz. Plant height (cm), Number of leaves ( $\text{plant}^{-1}$ ), Number of branches ( $\text{plant}^{-1}$ ), Number of pods ( $\text{plant}^{-1}$ ). All parameters of growth and yield of Black gram are found significant. The best treatment was found T<sub>9</sub> (P 100% + PSB culture) that showed the highest yield regarding, gave the best results with respect to plant height (56.43 cm), number of leaves  $\text{plant}^{-1}$  (46.20), number of branches  $\text{plant}^{-1}$  (8.00), test weight of 1000 seed (40.23 g), grain yield ( $8.58 \text{ q ha}^{-1}$ ) and straw yield ( $21.74 \text{ q ha}^{-1}$ ) respectively. The treatment was significantly higher as compared to other treatment combination. The economy of different treatment concerned, the treatment T<sub>9</sub> (P 100% + PSB culture) provides highest net profit of 62057.00 with cost benefit ratio is 1: 3.73 however, the minimum net profit of 41219.00 was recorded in the treatment T<sub>4</sub> (P 50% + un inoculated) with cost benefit ratio is 1:2.91.

**Keywords:** Black gram, Poultry manure, PSB, Phosphorus

### INTRODUCTION

Black gram (*Vigna mungo L.*) is one of the most important pulse crop grown in India. Black gram contributes 13% in total pulse area and 10% in total pulses production of India. Black gram seeds are highly nutritious containing higher amount of protein (24-26%) and are reported to be rich in potassium, phosphorus and calcium with good amount of sodium. It is also reported to be rich in vitamin A, B<sub>1</sub>, B<sub>3</sub> besides nutritionally rich protein, important minerals and vitamin. G. Selvakumar *et al.*, [1]

Its seeds are highly nutritious with protein (25-26%), carbohydrates (60%), fat (1.5%), minerals, amino acids and vitamins. Seed are used in the preparation of many popular dishes. It is one of the most important components in the preparation of famous south Indian dishes, e.g., dosa, idli, vada *etc.*, besides, it adds about 42 kg nitrogen per hectare in soil. (Source: Department of Agriculture, Govt. of Sikkim.). In India, Black gram is very popularly grown in Andhra Pradesh, Bihar, Madhya Pradesh, Maharashtra, Uttar Pradesh and West Bengal, Punjab, Haryana and Karnataka (Singh, 2010)., [2]

Phosphorus plays a vital role in the photosynthesis, respiration, cell division, cell enlargement and several other processes in living plants. Phosphorus, being an important component of storage and structural compounds of seed like phytin, phospholipids and nucleic acids. (Singh *et al.* 2012)., [3]

Levels of phosphorus are the most important factors affecting the yield of black gram. Madan Ananda

Jagannath *et al.*, [4].

Phosphate solubilizing micro organism used for treatment of seed or soil. The PSB like *Pseudomonas* and *Bacillus* also enhance the availability of phosphorus to plant by converting insoluble phosphorus from the soil into soluble form. Swati kadam *et al.*, [5]

Soil fertility cannot be maintained with the application of inorganic fertilizer alone. No single source can meet the increasing nutrient demands for agriculture, to achieve sustainability in production, there is a need to integrate both organic and inorganic source of nutrients. Punitha premanantharajan and komathy prapagar, [6].

For maintaining soil fertility poultry manure occupied a place as it is rich in nutrient then the other manures. Mohamad Ananullah *et al.*, [7].

### MATERIAL AND METHOD

The experiment was conducted during *Kharif* season 2016 on crop research farm of Department of Soil Science and Agricultural Chemistry, Naini Agricultural Institute, Allahabad. The area is situated on the south of Allahabad on the right side of the river Yamuna on the South of Rewa road at a distance of about 6 km from Allahabad city. It is situated at 25°02'23" N latitude, 81°05'38" E longitude and at the altitude of 98 meter above the sea level (MSL). The treatment consist of poultry manure and PSB Culture with different levels of Phosphorus. T<sub>1</sub> [P 0% + un inoculated (control)], T<sub>2</sub> [P 0% + poultry manure], T<sub>3</sub> [P 0% + PSB culture], T<sub>4</sub> [P 50% + un inoculated], T<sub>5</sub> [P 50% + poultry

\*Corresponding Author

manure]. T<sub>6</sub> [P 50% + PSB culture], T<sub>7</sub> [P 100% + un inoculated], T<sub>8</sub> [P 100% + poultry manure], T<sub>9</sub> [P 100% + PSB culture]. The trial was laid out in a randomized block design with three replications; plot size was 2 x 2 m for crop seed rate is 20 kg ha<sup>-1</sup> (*Vigna mungo* L.). Applies the recommended dose of nitrogen, potassium, poultry manure and PSB culture and phosphorus in different levels with source of urea, MOP, and SSP respectively, Basal dose of fertilizer was applied and poultry manure applies in respective plots according to treatment and PSB culture applies as seed treatment according to treatment. All the agronomic practices were carried out uniformly to raise the crop. And observation was recorded pre harvest viz, Plant height (cm), Number of leaf (plant<sup>-1</sup>), Number of branches (plant<sup>-1</sup>) and Number of pods (plant<sup>-1</sup>) at 15DAS, 30DAS, 45DAS and 60 DAS. Post harvest viz, Number of grains (pod<sup>-1</sup>), Test weight of 1000 seed (g), Grain yield (q ha<sup>-1</sup>) and Straw yield (q ha<sup>-1</sup>). Economics of different treatment viz, Cost of cultivation (Rs ha<sup>-1</sup>), Gross return (Rs ha<sup>-1</sup>), Net return (Rs ha<sup>-1</sup>) and Benefit cost ratio (B:C).

## RESULT AND DISCUSSION

All parameters found significant The best treatment was found T<sub>9</sub> (P 100% + PSB culture) that showed the highest yield regarding, gave the best results with respect to plant height (56.43 cm), number of leaves plant<sup>-1</sup> (46.20), number of branches plant<sup>-1</sup> (8.00), test weight of 1000 seed (40.23 g), grain yield (8.58 q ha<sup>-1</sup>), straw yield (21.74 q ha<sup>-1</sup>) and highest net profit of 62057.00 with cost benefit ratio is 1: 3.73. The treatment was significantly higher as compared to other treatment combination.

Increase in plant height, number of leaf and number of branches due to increasing level of phosphorus fertilizer and Bio- inoculation (PSB culture and poultry manure) may be due to adequate or nutrients which is turns help in vigorous vegetative growth of

plants and subsequently increase the plant height, number of leaf and number of branches through cell elongation cell division photosynthesis and turbidity of plant cell. The increase in nodulation and phosphorus fixation due to inoculation of phosphorus solubilizing bacteria leads to more plant height. Similar results about plant height, number of leaf and number of branches also reported by Shanti *et al.* (2008) and R.P Singh *et al.* (2008), [8] & [9].

The probable reason for number of pods plant<sup>-1</sup>, number of grains pod<sup>-1</sup> test weight 1000 seed (gm) may be due to beneficial effect phosphorus fertilizer and Bio- inoculation (PSB culture and poultry manure). similarly, also reported by Marko *et al.* (2013), [10].

The factor which are directly responsible for ultimate grain production pods plant<sup>-1</sup>, grains pod<sup>-1</sup>, 1000 grain weight were increased almost significantly due to increased supply of phosphorus fertilizer and Bio- inoculation (PSB culture and poultry manure). Available N content significantly increased may be due to fact that legumes contribute to the total pool of nitrogen in the soil as observed by Ahmad *et al.* [11]

Poultry manure additions up to 50 t ha<sup>-1</sup> improved soil organic matter total N and available P as well as improved soil physical properties as indicated by reduction in soil bulk density and increased in soil moisture content Ewulo *et al.* [12].

Application of organic manure increased the supply of easily assimilated major as well as micro nutrient to plants, besides mobilizing, unavailable nutrients in to available from more over, bio-inoculation also perform better when soil is well supplied with nutrients. Similar results about grain yield (q ha<sup>-1</sup>) also reported by Datt *et al.* (2003); Naseen and Farid (2003), [13] & [14]

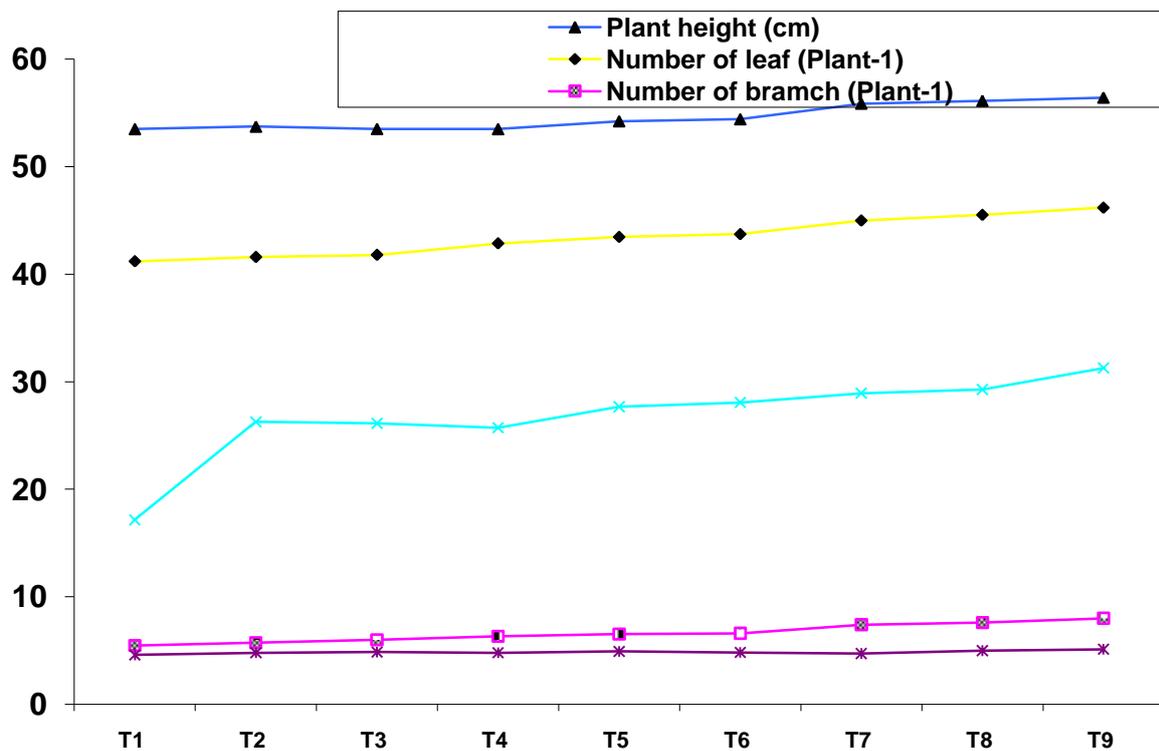
The results in given Table indicates some of the important parameter on growth and yield of black gram crop different treatment of poultry manure and PSB culture with levels of phosphorus.

**Table 1.** Effect of poultry manure and PSB Culture With different levels of phosphorus on growth of Black gram crop.

Treatment	Plant height (cm)	Number of leaf (Plant <sup>-1</sup> )	Number of branch (Plant <sup>-1</sup> )	Number of pod (Plant <sup>-1</sup> )	Number of grain (pod <sup>-1</sup> )
T <sub>1</sub>	53.50	41.20	5.47	17.17	4.60
T <sub>2</sub>	53.73	41.60	5.73	26.27	4.80
T <sub>3</sub>	53.51	41.80	6.00	26.13	4.87
T <sub>4</sub>	53.50	42.87	6.33	25.73	4.80
T <sub>5</sub>	54.23	43.47	6.53	27.67	4.93
T <sub>6</sub>	54.42	43.73	6.60	28.07	4.87
T <sub>7</sub>	55.87	45.00	7.40	28.93	4.73
T <sub>8</sub>	56.13	45.53	7.60	29.27	5.00
T <sub>9</sub>	56.43	46.20	8.00	31.27	5.13
<b>S. Em (±)</b>	<b>0.35</b>	<b>0.11</b>	<b>0.11</b>	<b>3.44</b>	<b>0.15</b>
<b>C. D. at 5%</b>	<b>0.06</b>	<b>0.24</b>	<b>0.23</b>	<b>7.29</b>	<b>0.32</b>

**Table 2.** Effect of poultry manure and PSB Culture With different levels of phosphorus on yield attributes and economic of Black gram crop.

Treatment	Test weight 1000 seed <sup>-1</sup> (gm)	Grain yield (q ha <sup>-1</sup> )	Straw yield (q ha <sup>-1</sup> )	BCR
T <sub>1</sub>	39.17	6.25	15.82	3.02
T <sub>2</sub>	39.27	7.30	18.49	2.72
T <sub>3</sub>	39.90	7.06	17.64	3.40
T <sub>4</sub>	39.87	6.35	16.29	2.91
T <sub>5</sub>	39.90	7.18	18.21	2.57
T <sub>6</sub>	40.83	7.09	17.93	3.24
T <sub>7</sub>	39.97	7.94	20.01	3.45
T <sub>8</sub>	40.00	8.47	21.28	2.91
T <sub>9</sub>	40.23	8.58	21.74	3.73
S. Em (±)	<b>0.33</b>	<b>0.08</b>	<b>0.45</b>	
C. D. at 5%	<b>0.70</b>	<b>0.18</b>	<b>0.95</b>	



**Fig:** Effect of poultry manure and PSB culture With different levels of phosphorus on Plant height, Number of leaves, Number of branches, Number of pods Plant<sup>-1</sup>, Number of grains Pod<sup>-1</sup> of black gram crop.

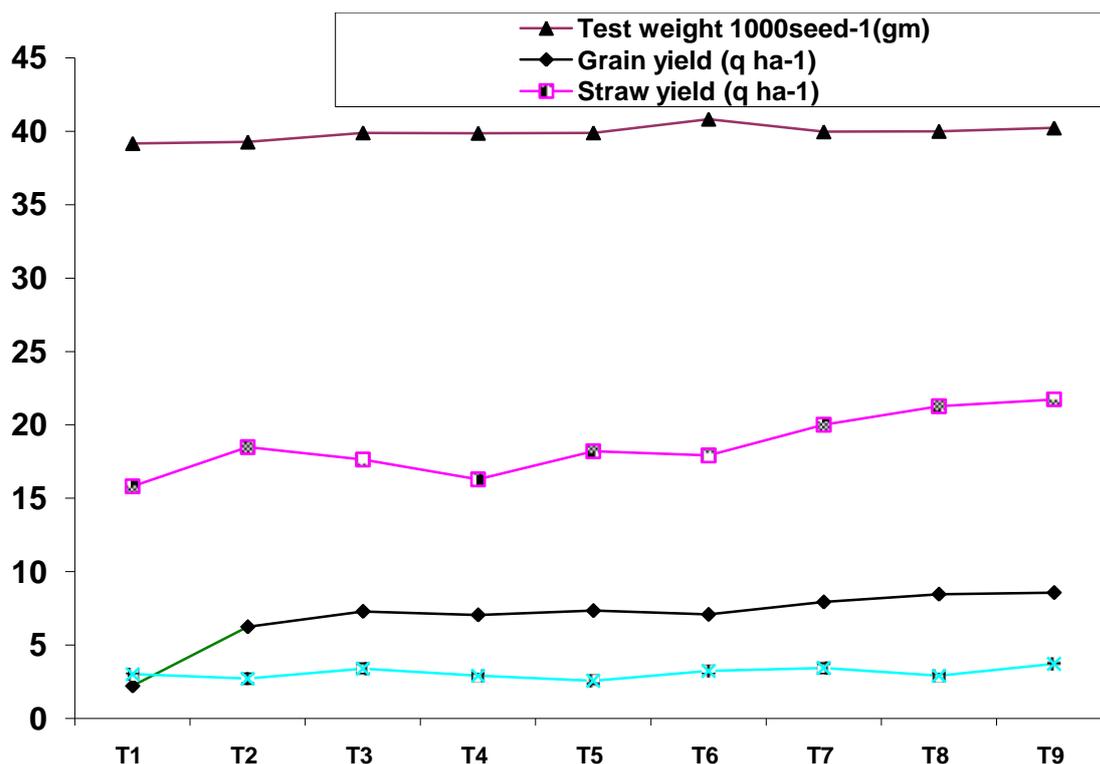


Fig: Effect of poultry manure and PSB culture With different levels of phosphorus on Test weight of 1000seed, Grain yield, Straw yield and Benefit Cost Ratio of black gram crop

## CONCLUSION

It may be concluded from trial that the different level of phosphorus with PSB culture in the experiment. The best treatment was found T<sub>9</sub> (P 100% + PSB culture) that showed the highest yield regarding, gave the best results with respect to plant height (56.43 cm), number of leaves plant<sup>-1</sup> (46.20), number of branches plant<sup>-1</sup> (8.00), test weight of 1000 seed (40.23 g), grain yield (8.58 q ha<sup>-1</sup>), straw yield (21.74 q ha<sup>-1</sup>) and highest net profit of 62057.00 with cost benefit ratio is 1: 3.73.

## ACKNOWLEDGEMENT

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## IMPACT ANALYSIS OF CLUSTER FRONTLINE DEMONSTRATION IN SOYBEAN CULTIVATION

R.K. Dwivedi<sup>1</sup>, B.K. Tiwari<sup>2\*</sup> and K.S. Baghel<sup>2</sup>

<sup>1</sup>JNKVV Krishi Vigyan Kendra, Damoh (M.P.)

<sup>2</sup>JNKVV Krishi Vigyan Kendra, Rewa (M.P.)

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**Abstract:** The domestic requirement of oilseed had been manifold of a modern living standard which has been fulfilled through the imports that leads to imbalance the Indian economy. To fulfill the domestic demand and to boost the production and productivity, cluster frontline demonstrations (CFLDs) on Soybean were conducted at farmer's field through Krishi Vigyan Kendra Damoh (M.P.). These demonstrations were conducted in two villages namely Jortala and Bamori during kharif seasons of 2015-16 and 2016-17. The results of CFLDs show a greater impact on farming community due to significant increase in crop yield greater than farmer practice. The economics and benefit cost ratio of both farmers practice (FP) and recommended practice (RP) were worked out. An average of Rs. 30990/ha was recorded net profit under RP while it was Rs. 18792/ha under FP. Benefit cost ratio was 2.10 under RP, while it was 1.87 under FP. By introducing the proven technology i.e improved variety (JS 95-60), seed treatment, sowing in broad bed furrow method, integrated weed management, recommended dose of fertilizers on soil test base and integrated pest management by encouraging the farming community of the district through recommended technologies were followed in the CFLDs.

**Keywords:** Soybean, CFLDs, Farming community, Net profit, B:C ratio

### INTRODUCTION

Soybean (*Glycine max* (L) merrill) belongs to family leguminaceae. It is mainly grown in Kharif Season and occupies second place followed by ground nut. Production of soybean in India is dominated by Maharashtra and Madhya Pradesh which contribute 89% of the total production. Madhya Pradesh and Maharashtra have 45% and 40% share in production respectively; Anonymous (2017).

Among the Kharif oilseeds, soybean is an important crop in India and is mostly grown under rainfed condition. The average productivity of soybean is 950 kg/ha in Madhya Pradesh (2016-17), which is very low as compared to national productivity (1450 kg/ha). The reasons for low productivity were traditional method of cultivation. With the development of high yielding varieties and use of improved management practices, there is a much scope for further increase in yield. So the Department of Agriculture, Cooperation and Farmers Welfare (DAC&FW) had sanctioned the project "Cluster Frontline Demonstration on oilseed to ICAR-ATARI Jabalpur. This project was implemented in Krishi vigyan kendras of Zone IX with main objective to boost the production and productivity of soybean through frontline demonstration (FLDs) with latest and specific technologies.

### MATERIAL AND METHOD

Field demonstrations were conducted under close supervision of krishi vigyan Kendra, Damoh. Total 100 cluster front line demonstrations under real farming situations were conducted during kharif season of 2015-16 and 2016-17 at two different villages namely; Jortala and Bamori, respectively

under krishi vigyan kendra operational area. The area under each demonstration was 0.4 ha. The soil was sandy clay-loam in texture with moderate water holding capacity, low in organic carbon (0.2-0.42%), low in available nitrogen (96.5-146.9 kg/ha), low to medium in available phosphorus (8.0 -12.6 kg/ha), low in available potassium (170.2-225.2 kg/ha) and soil pH was slightly acidic to neutral in reaction (6.4-7.0). The treatment comprised of recommended practice (Improved variety JS-95-60, integrated nutrient management-@ 20:60:40:20 kg NPKS/ha + *Rhizobium* + PSB @ 5 g/kg seed, integrated pest management- deep ploughing + seed treatment with *Trichoderma viridae* @ 5 g/kg seed + indoxacarb @ 500 ml/ha etc. vs. farmers practice. Deep ploughing was done during the April month. Crop was sown between 25 June to 10 July with a spacing of 45 cm and seed rate was 75 kg/ha. An entire dose of N and P through diammonium phosphate, K through muriate of potash and sulphur through ZnSO<sub>4</sub> was applied as basal before sowing. The seeds were treated with *Trichoderma viridae* @5 g/kg seeds then inoculated by *Rhizobium* and phospho-solubilizing bacteria biofertilizers each 5g/kg of seeds. Application of Imazethapyr @100 g a.i./ha at 25-30 DAS followed by slight hand weeding at 45 DAS for effective weed management was done; used flat fan nozzle. One spray of Indoxacarb @500 ml/ha with 500 liters of water was given at the time of incidence of stem fly. The crop was harvested between 25<sup>th</sup> October to 10<sup>th</sup> November during both years of demonstrations. Farmer's practice constituted there were no deep ploughing was done during summer, old seed of variety JS-335 was used, crop was sown on the same time of demonstration, broadcasting method of sowing, higher seed rate (100 kg/ha) sown, imbalance dose of fertilizers applied (15:40:0 kg NPK/ha), no

\*Corresponding Author

seed treatment, no biofertilizers, no plant protection measures and one hand weeding at 30-35 DAS were adopted. Crop was harvested on the same time of harvesting of demonstration plots. Harvesting and threshing operations done manually; 5m x 3m plot harvested in 3 locations in each demonstration and average grain weight taken at 14% moisture. Similar procedure adopted on FP plots under each demonstration then grain weight converted into quintal per hectare (q/ha). Before conduct the demonstration training to farmers of respective villages was imparted with respect to envisaged technological interventions. All other steps like site selection, farmers selection, layout of demonstration, farmers participation etc. were followed as suggested by Choudhary (1999). Visits of farmers and extension functionaries were organized at demonstration plots to disseminate the technology at large scale. The gross returns, cost of cultivation, net returns and benefit cost ratio (B:C ratio) were calculated by using prevailing prices of inputs and outputs and finally the extension gap, technology gap and technology index were worked out. To estimate the technology gap, extension gap and technology index, following formulae given by Kadian *et al* (1997) have been used.

$$\text{Technology Index} = \frac{(P_i - D_i) \times 100}{P_i}$$

Where,

P<sub>i</sub>- Potential yield of i<sup>th</sup> crop

D<sub>i</sub>- Demonstration yield of i<sup>th</sup> crop

## RESULT AND DISCUSSION

### Yield attributing parameters:

The yields attributing parameters like number of pods/plant and branches/plant of soybean obtained over the years under recommended practice as well as farmers practice are presented in Table 1. The Number of pods/plant of soybean with mean of 65 under recommended practice on farmer's field as against 43 recorded under farmers practice. Similarly higher number of branches/plant was recorded under recommended practice 7 as compared to farmers practice mean of 4. The higher values of number of pods/plant and number of branches/plant following recommended practice as well as farmers practice was due to the use of latest high yielding variety, integrated nutrient management and integrated pest management on soybean during both the years of demonstration. Similar results have been reported earlier by Prasad (2005).

### Seed yield:

The yields of soybean obtained over the years under recommended practice as well as farmers practice are presented in Table 1. The productivity of soybean average of 19 q/ha under recommended practice on farmers field as against a mean yield of 12.8 q/ha recorded under farmers practice. The higher productivity following recommended practice as well

as farmers practice was during both the years which might be due to congenial climate for better growth of crop. The higher yield of soybean under recommended practices was due to the use of latest high yielding variety, integrated weed management, integrated nutrient management and integrated pest management. Similar results have been reported earlier by Jain and Dubey (1998).

### Economics:

The inputs and outputs prices of commodities prevailed during both the year of demonstrations were taken for calculating cost of cultivation, net returns and benefit cost ratio (Table 3). The investment on production by adopting recommended practices ranged from Rs.27416 to 28718/ha with a mean value of Rs.28067/ha against farmers practice where the variation in cost of production was Rs. 22175- Rs. 20720/ha, mean of Rs. 21447/ha. Cultivation of soybean under recommended practices gave higher net return of Rs.32784 and Rs.29197/ha compared to Rs.19341 and Rs.18252/ha under farmers practice during 2015-16 and 2016-17, respectively. The average benefit cost ratio of recommended practices was 2.10, varying from 2.01 to 2.19 and that of farmers practice was 1.87. This may be due to higher yields obtained under recommended practices compared to farmers practice. Similar results have been reported earlier on chickpea by Tomar *et al* (1999) and Tomar (2010).

### Extension and technology gap

The extension gap ranging between 6.2 q/ha during the period of study emphasized the need to educate the farmers through various means for the adoption of improved agricultural production to reverse the trend of wide extension gap (Table-2). The trend of technology gap ranging between 5.6 – 6.3 q/ha reflected the farmer's cooperation in carrying out such demonstration with encouraging results in both the years. The technology gap observed may be attributed to the dissimilarity in weather conditions. The technology index showed the feasibility of the evolved technology at the farmer's field. The lower the value of technology index, the more is the feasibility of the technology. As such, the reduction in technology index from 22.4% during 2015-16 to 25.2% during 2016-17 exhibited the feasibility of the demonstrated technology in this region.

### HRD Components

To increase the understanding and skill of the district and village about improved cultivation technology of soybean, various training programme, ratio talk, field day. Kisan sangosthi, CD shows, folders and kisan mela were organized both at district level and village level (Table-4). These human resource development (HRD) components not only helped in proper understanding of technology required to adopt farmers themselves compared in actual RP with FP (Table4).

### Constraints observed during CFLDs

The farmer's yields were affected by various environmental and socio-economic factors like non availability of quality seed, unawareness in seed treatment, use of recommended dosage of fertilizer

etc. high losses in yield observed due to heavy infestation of girdle beetle, stem fly, white fly and pod borer due to improper method and time of application of pesticide.

**Table 1.** Performance of CFLDs on soybean as affected by recommended practices as well as farmers practices (Mean of two years 2015-16, 2016-17).

S.No	Parameters	Treatment	
		Recommended Practice (RP)	Farmers Practice (FP)
1.	No of Branches/Plant	7.0	4.0
2.	No of Pods/Plant	65	43
3.	Seeds/Pod	3.5	2.0
4.	Grain yield q/ha	19.0	12.8

**Table 2.** Productivity, Technology gap, Extension gap and technology index of soybean as affected by recommended practice as well as farmers Practices.

Year	Area (ha)	No. of Demo.	Grain yield q/ha			% increase over FP	Technology Gap (q/ha)	Extension gap q/ha	Technology Index (%)
			Potential	RP	FP				
2015-16	20	50	25	19.4	13.2	46.9	5.6	6.2	22.4
2016-17	20	50	25	18.7	12.5	49.6	6.3	6.2	25.2
<b>Total/ Mean</b>	<b>40</b>	<b>100</b>	<b>25</b>	<b>19.0</b>	<b>12.8</b>	<b>48.2</b>	<b>5.9</b>	<b>6.2</b>	<b>23.8</b>

**Table 3.** Economics of front line demonstration of Soybean as affected by recommended Practice as well as farmer's Practices.

Year	Yield (q/ha)		% increase over FP	Gross Expenditure (Rs/ha)		Gross Return (Rs/ha)		Net Return (Rs/ha)		B:C Ratio	
	RP	FP		RP	FP	RP	FP	RP	FP	RP	FP
2015-16	19.4	13.2	46.9	27416	22175	60200	41516	32784	19341	2.19	1.87
2016-17	18.7	12.5	49.6	28718	20720	57915	38972	29197	18252	2.01	1.88
<b>Mean</b>	<b>19.0</b>	<b>12.8</b>	<b>48.2</b>	<b>28067</b>	<b>21447</b>	<b>59057</b>	<b>40244</b>	<b>30990</b>	<b>18792</b>	<b>2.10</b>	<b>1.87</b>

**Table 4.** HRD Component : Cumulative data of 2015-16 and 2016-17.

S.No	HRD Component	Frequency	Beneficiaries
1.	Training	10	565
2.	Radio Talk	1	Mass
3.	CD Shows	4	252
4.	Kisan Mela	2	3217
5.	Kisan Sangosthi	4	105
6.	News Paper Coverage	11	Mass
7.	Folder	1	Mass
8.	Kisan Mobil Sandesh	6	14672

## CONCLUSION

Cluster frontline demonstration of oilseed (soybean) conducted in two village in Damoh district and result concluded the average yield 19.0q/ha in RP as compared to 12.8q/ha in FP. It was observed that potential yield can be achieved by imparting scientific knowledge to the farmers, providing the quality need based inputs and proper application of inputs. Horizontal spread of improved technology may be achieved by the successful implementation of frontline demonstration and various extension activities in farmer's field. For wide dissemination of technologies recommended by SAUs and other research institute, more number of FLDs should be conducted.

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