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DEVLOPMENT OF HIGHER EFFICIENT MUTANT OF BEAUVERIA BASSIANA WITH ENFLUENCE OF UV LIGHT

Solanki Seema[1], Tandon Neeraj [2]

1. Shri Jagdishprasad Jhabarmal Tibrewala University, Jhunjhunu, Rajasthan, India

2. Scientific and Applied Research Center, Meerut, U.P, India

ABSTRACT: UV light has great significant potential to create a mutant microbial species and some may increase or decrease the efficiency of Microbial species. UV light have used as causative agent for create the mutation in *Beauveria bassiana* and it's converted in mutant *beauveria bassiana.* UV light exposure time has recorded 90 minutes for the conversation in it. ITS 4 oligonucleotide primer and ITS 6 oligonucleotide primer Sequence used for multiple sequencing. 18S ribosomal RNA gene used for identification of strain, sequence internal transcribed spacer 4 and Internal transcribed spacer 6 in *Mutant Beauveria bassiana* and wild *Beauveria bassiana*. *Beauveria bassiana* bentonite oil based formulations can same as based spectrum biological control against Helicoverpa armigera, aphids & thrips in Soybean, Tover & Mung crop. Oil based Bentonite is excellent carrier material for *Beauveria bassiana* strain and also effective economical cost with great shelf life of bacteria which maintained biological action for 2 years. *Beauveria bassiana* Bentonite oil base liquid formulation has been required one week for infection on larva of each. Significant control of mutant *Beauveria bassiana* showed by 2×10^9 spore per ml around 73-80% observed, increasing dose of *Beauveria bassiana* showed failed to control of insect.

KEYWORDS: converted, exposure, increasing, efficiency, internal transcribed spacer, oligonucleotide primer

***Corresponding Author: Solanki Seema**

Shri Jagdishprasad Jhabarmal Tibrewala University Rajasthan

* Email Address: seema.solanki66@gmail.com

1.INTRODUCTION

In recent scenario chemical pesticides is big problem in agriculture farming. Due to chemical pesticides many beneficial microorganism dies, also cause harmful effect on human health, environment, soil, air and water. Many pest insect developed resistance towards chemical pesticides. This problem may be control by using of Entomopathogenic fungi instead place of chemical pesticides. 700 Entomopathogenic fungi have been searched and around 90 genera are pathogenic for insect pest. [1]These Entomopathogenic fungi are using as bio-pesticides and showing good result against pest but it is not using at desire level in the world. It has potential to displace the dominance of chemicals. Entomopathogenic fungi have great potential for the controlling of insect and pest population it they used in proper way and it should be used as initial as field preparation, seed treatment, root treatment and foliar spray. If they have used in proper manner defiantly found excellent control in insect pest population also they control many disease which caused by plant pathogeng and soil harmful fungi in all crop. They are ecofriendly; also do not cause any harmful effect on humanity, environmentfriendly, air, soil and also is farmers friends. Entomopathogenic fungi *Beauveria bassiana* significantly control have reported against trips, white fly and Helicoverpa armigera with bentonite oil base formulation. *Beauveria bassiana* has broad countenance of executive insect pest. 280-320 nm UV radiation caused assured mutation in microorganism. Mutant *Beauveria bassiana* has obtained with ultra violet radiation in 90 minutes and 10cm distance between uv light and fungus. Efficacy and potential has been increased by mutation against insect it's observed via experiment. Mutation some time cause positive or negative feature in microorganism. Positive mutation in *Beauveria bassiana* may control 90% population of insect in a week. Spore of mutant *Beauveria bassiana* strain also live at 50 degree centigrade in flied and native *Beauveria bassiana* strain dies gradually up temperature up to 45 degree centigrade. [2]. *Beauveria bassiana* released beauvericin and mycotoxin in insect body which caused mortality effect to the insect. These toxins have been documented to be most larvacidal efficacy nature. Helicoverpa armigera has found common insect in gram pod borer. It`s recorded serious pest problem in gram crop. Beavericin examined in saliva gland of 10-8 day's larva of Helicoverpa armigera. Mycotoxin of Beauveria bassian in Helicoverpa armigera has reported after 24 hrs treatment, the dosage of this toxin was 0.147 micron gm/gm body weight (LD_{50}). Pest investigation was carried out to highlight for farmer awareness which should be going to stop using of chemical pesticide. [3] Farmer should be promoted for the use of Entomopathogenic, created the awareness against chemical pesticides that leads to prevent our soil and air system. Entomopathogenic fungus *Beauveria bassiana* has great significance to effectively control pest & insect in crop. Optimization growth of *Beauveria bassiana* strain has been obtained bioassay

of different formulation for their efficacy with crop application with laboratory and field trials. The investigation has established that the mutant *Beauveria bassiana* strain has worked as effectively biological control against Helicoverpa armigera, Aphids & Thrips. (4)

2.MATERIALS AND METHODS

A. Sample areas were selected and collected for the isolation of *Beauveria bassiana.*

B. Isolation of EPF from sample with serial dilution methods.

Materials- Samples, sterile distilled water, Martin Rose Bangle agar.

Procedure

1. 1 gm sample suspension was prepared in 10 ml distilled water and mixed properly, allowed to settling down and performed serial dilution.
2. Loopful suspension was taken from supernatant of last three aliquots and streaked on Martin Rose Bangle agar.
3. Petri plates have incubated at room temperature for seven days.
4. Colonies characters have observed on Petri plate and prepared mount slide with the help of Lacto phenol cotton blue and observed in microscope.

C. **Ultra light exposure time interval for *Beauveria bassiana.*** Following time intervals have used for causing mutatin in *B.bassiana*.

Table 1.Ultra light exposure time interval on *Beauveria bassiana*.

S.no	Ultra light exposure time	*Beauveria bassiana*
1.	15 minute	Bb-1
2.	30 minute	Bb-2
3.	60 minute	Bb-3
4.	90 minute	Bb-4
5.	120 minute	Bb-5

After UV light exposure *Beauveria bassiana* culture have transferred in fresh media, incubated for 8 days after observed the physical characteristics of fungi and compared to each others. When the changes were found in physical characteristics of each other go through DNA isolation of both fungi.

D. Materials and Methods for Fungal DNA isolation for Normal *Beauveria bassiana* and *Mutant Beauveria bassiana*.

Steps-

- **Isolation of genomic DNA from Normal *Beauveria bassiana* -** N- Cetyl- N, N, N-trimethyl- ammonium bromide (CTAB) method have followed for Total genomic DNA isolaton from the Normal *Beauveria bassiana* and Mutant *Beauveria bassiana* fungi.
- **Following Chemicals and reagents used in** DNA **isolation method that's discussed in below table.**

1. Extraction buffer have consumed for DNA isolation.

Table 2. Extraction buffer used in Fungal DNA isolation

	Stock solution	**Buffer composition**
1.	**1 M Tris HCl**	100 mM Tris HCl
2.	**1M EDTA**	100 mM EDTA
3.	**4 M NaCl**	1.4 M NaCl
		1% CTAB
		Proteinase K - 0.03µg/µl

2. SDS 20% w/v
3. Chloroform: isoamyl alcohol (24:1)
4. Isopropanol
5. Ethyl alcohol 70% v/v

E. Procedure for DNA isolation

- 0.5 g Fungal Mycelium was taken and grinded with 25 mg PVPP using mini grinder instrument and then centrifuge at 10000 rpm 2 min. at 4^{o}C.
- Pellet washed with sterile distilled water and Centrifuged at 1000rpm 20 min. at 4^{o}C.
- 675 µl of extraction buffer was added and incubated at 37°C for 30 min.
- 75µl of SDS (20%) was added and incubated at 65°C for 2 hours.
- Centrifuged at 10000 rpm for 10 min at 4°C
- Clear solution was collected in a sterile microcentrifuge tube.
- Equal volume of Phenol: chloroform: isoamyl alcohol (25:24:1) was added.
- Centrifuged at 10000 rpm for 10 min. at 4°C
- Equal volumes of Chloroform: Isoamyl alcohol (24:1) was added.
- Centrifuged at 10000 rpm for 10 min. at 4°C
- The aqueous phase was removed and taken in a sterile microcentrifuge tube.

- 0.6 volumes of isopropyl alcohol was added and incubated at room temperature for 1hour.
- Centrifuged at 10000 rpm for 10 min.
- Pellet was washed in 500µl of 70% ethanol.
- Centrifuged at 10000 rpm for 10 min at room temperature.
- Pellet was dried and dissolved in 20 µl sterile distilled water.

D. Quantification of Isolated DNA

The quantity of the isolated DNA was checked in UV-VIS spectrophotometer (VivaspecBiophotometer, Germany). From the stock 1µl DNA was mixed with 49-µl sterile distilled water to get 50 times dilution. The A260/A280 ratio was recorded to check the purity of DNA preparation.

E. PCR Amplification

Reagents and the optimal PCR reaction mixture -PCR amplification of ITS region was done in 20 µl of reaction mixture containing PCR buffer, 1X (Kappa, SA); $MgCl_2$, 3 mM; dNTP mix, 0.25 mM; *Taq*DNA polymerase, 0.05 U; primer, 1 picomol and template DNA, 50 ng. Sterile nuclease free water is used as negative control. Bellowed PCR components have used for PCR of formation DNA copies.

Table 3.PCR components used in PCR reaction

	PCR components	PCR components
1.	Nuclease free water	10.75
2.	10X reaction buffer with $MgCl_2$ (1.5mM)	2.00
3.	dNTP mix (2.5mM)	2.00
4.	Primer ITS 4 (10picomoles/ µl)	2.00
5.	Primer ITS 6 (10picomoles/ µl)	2.00
6.	*Taq* DNA polymerase (5U)	0.25
7.	Template DNA (50ng/ µl)	1.00
8.	Template DNA (50ng/ µl)	1.00

Table 4. PCR temperature profile have used PCR reactions.

	Initial denaturation	94°C for 2 min
	Denaturation	94°C for 50 s
	Annealing	48°C for 30 s } 30 cycles
	Extension	72°C for 1min30S
	Final extension	72°C for 6 min

Table 5. Following Oligonucleotide primers used in I6RNA amplification.

	Oligonucleotide	**Sequences (5'- 3')**	**GC %**	**Tm Value**	**Length**	**Product Size**
1.	**ITS 4**	TCC TCC GCT TAT TGA TAT G	50	51.0 ^{0}C	19	700 bp
2.	**ITS 6**	GAA GGT GAA GTC GTA ACA AGG	60	56.0 ^{0}C	21	

3.RESULTS AND DISCUSSION

Normal *Beauveria bassiana has* converted in mutant Beauveria bassiana with the exposure of Ultra Violet light, positive mutation has occurred in it that's increased efficiency to cause infection in pest or insect. The exposure time of Ultra Violet light was given 90 minutes on *Beauveria bassiana*. In 90 minutes Wild strain of *Beauveria bassiana Tn-Bb-001* converted into *Beauveria bassiana strain GZGY-1*-3 with 10 cm distance used between B.bassina and UV light.

Normal Beauveria bassiana- *Beauveria bassiana* is isolated from the biopesticides products of Krishi Bio-products and Research Rau, Indore M.P region soil. It has limiting survival of condia of *Beauveria bassiana* in epigial habitats appear to be sunlight. Extremely major different have reported between in both species with various parameters. The image of normal Beauveria bassiana have bellow image. After isolation of normal *Beauveria bassiana*, Transferred in fresh culture media, Incubation, Observation, Interpretation, DNA isolation, Quantification, PCR, and bioinformatical analyses have performed for both fungal species. All above methods have used for the identification of *Normal Beauveria bassiana.*

Fig.1 Image of Normal *Beauveria bassiana*

N- Cetyl- N, N, N-trimethyl- ammonium bromide (CTAB) method have followed for total genomic DNA isolaton from the Normal *Beauveriabassiana* and Mutant *Beauveriabassiana* fungi..The quantity of the isolated DNA was checked in UV-VIS spectrophotometer

(VivaspecBiophotometer, Germany), from the stock 1µl DNA was mixed with 49-µl sterile distilled water to get 50 times dilution. The A260/A280 ratio was recorded to check the purity of DNA preparation.

Gel data obtained by electrophoresis

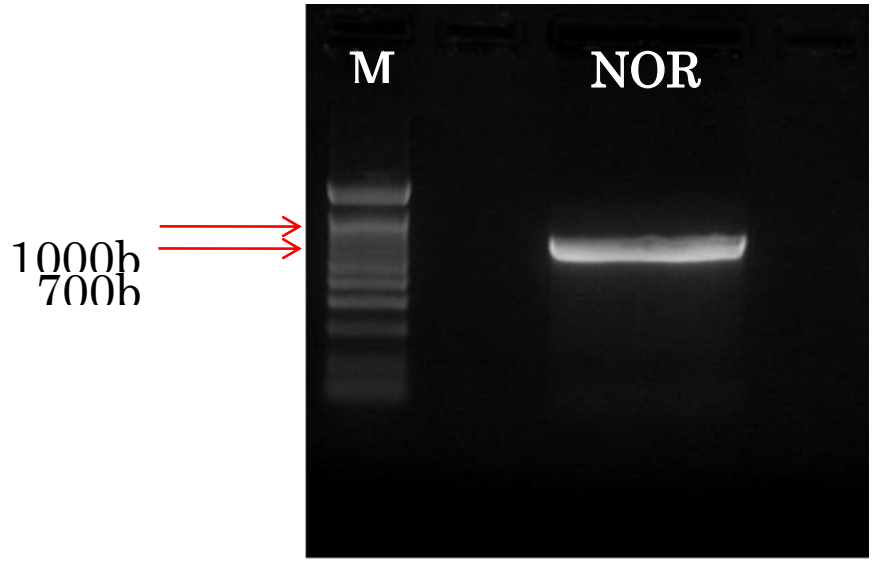

Fig.2. Normal *Beauveria bassiana* band obtained on electrophoresis gel

PCR Amplification

Reagents and the optimal PCR reaction mixture

PCR amplification of ITS region was done in 20 µl of reaction mixture containing PCR buffer, 1X (Kappa, SA); $MgCl_2$, 3 mM; dNTP mix, 0.25 mM; *Taq*DNA polymerase, 0.05 U; primer, 1 picomol and template DNA, 50 ng. Sterile nuclease free water is used as negative control.

PCR Amplification was done and obtains multiple (685BP) sequence alignment.

GGTCTATAGGTTCACAGAAGGGTAGGGAGTTGAAACTCGGTAATGATCCCTCCGC
TGGTCACCAACGGAGACTTGTGTTGATGAGACGCGGAGGGACATTACCGAGTTTT
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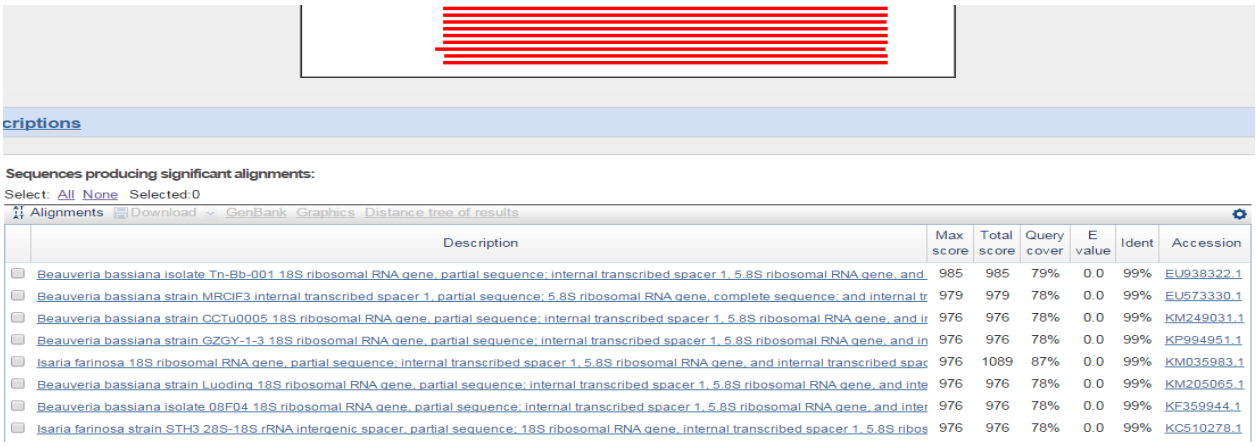

criptions

Sequences producing significant alignments:

Select: All None Selected:0

Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Beauveria bassiana isolate Tn-Bb-001 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and	985	985	79%	0.0	99%	EU938322.1
Beauveria bassiana strain MRCIF3 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal tr	979	979	78%	0.0	99%	EU573330.1
Beauveria bassiana strain CCTu0005 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and in	976	976	78%	0.0	99%	KM249031.1
Beauveria bassiana strain GZGY-1-3 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and in	976	976	78%	0.0	99%	KP994951.1
Isaria farinosa 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spac	976	1089	87%	0.0	99%	KM035983.1
Beauveria bassiana strain Luoding 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and inte	976	976	78%	0.0	99%	KM205065.1
Beauveria bassiana isolate 08F04 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and inter	976	976	78%	0.0	99%	KF359944.1
Isaria farinosa strain STH3 28S-18S rRNA intergenic spacer, partial sequence; 18S ribosomal RNA gene, internal transcribed spacer 1, 5.8S ribos	976	976	78%	0.0	99%	KC510278.1

Fig. 3 Image of multiple alignment sequencing

CLUSTAL O(1.2.1) multiple sequence alignment obtained by Next generator sequencer.

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gi|197365659|Beauveria      ------------------------------------------------------------
NORMAL-BB                       GGTCTATAGGTTCACAGAAGGGTAGGGAGTTGAAACTCGGTAATGATCCCTCCGCTGGTC
gi|190335354|Beauveria      ------------------------------------------------------------
gi|684907091|Beauveria      ------------------------------------------------------------
gi|916346079|Beauveria      ------------------------------------------------------------
gi|699977277|Isaria        ------------------------------------------------------------
gi|699210027|Beauveria      ------------------------------------------------------------

gi|197365659|Beauveria      ------------------TGATGACAGCGGAGGGACATTACCGAGTTTTCACTCCCTAA
NORMAL-BB                       ACCAACGGAGACTTGTGTTGATGAGACGCGGAGGGACATTACCGAGTTTTCACTCCCTAA
gi|190335354|Beauveria      -------------------------GCGGAGGGACATTACCGAGTTTTCAACTCCCTAA
gi|684907091|Beauveria      -------------------------GCGGAGGGATCATTACCGAGTTTTCAACTCCCTAA
gi|916346079|Beauveria      -------------------------GCGGAGGGATCATTACCGAGTTTTCAACTCCCTAA
gi|699977277|Isaria        -------------------------GCGGAGGGATCATTACCGAGTTTTCAACTCCCTAA
gi|699210027|Beauveria      -------------------------GCGGAGGGATCATTACCGAGTTTTCAACTCCCTAA
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gi|684907091|Beauveria      CCCTTCTGTGAACCTACCTATCGTTGCTTCGGCGGACTCGCCCCAGCCCGGACGCGGACT
gi|916346079|Beauveria      CCCTTCTGTGAACCTACCTATCGTTGCTTCGGCGGACTCGCCCCAGCCCGGACGCGGACT
gi|699977277|Isaria        CCCTTCTGTGAACCTACCTATCGTTGCTTCGGCGGACTCGCCCCAGCCCGGACGCGGACT
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gi|684907091|Beauveria      GGACCAGCGGCCCGCCGGGGACCTCAAACTCTTGTATTCCAGCATCTTCTGAATACGCCG
gi|916346079|Beauveria      GGACCAGCGGCCCGCCGGGGACCTCAAACTCTTGTATTCCAGCATCTTCTGAATACGCCG
gi|699977277|Isaria           GGACCAGCGGCCCGCCGGGGACCTCAAACTCTTGTATTCCAGCATCTTCTGAATACGCCG
gi|699210027|Beauveria      GGACCAGCGGCCCGCCGGGGACCTCAAACTCTTGTATTCCAGCATCTTCTGAATACGCCG
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gi|197365659|Beauveria      CAAGGCAAAACAAATGAATCAAAACTTTCAACAACGGATCTCTTGGCTCTGGCATCGATG
NORMAL-BB                     CAAGGCAAAACAAATGAATCAAAACTTTCAACAACGGATCTCTTGGCTCTGGCATCGATG
gi|190335354|Beauveria      CAAGGCAAAACAAATGAATCAAAACTTTCAACAACGGATCTCTTGGCTCTGGCATCGATG
gi|684907091|Beauveria      CAAGGCAAAACAAATGAATCAAAACTTTCAACAACGGATCTCTTGGCTCTGGCATCGATG
gi|916346079|Beauveria      CAAGGCAAAACAAATGAATCAAAACTTTCAACAACGGATCTCTTGGCTCTGGCATCGATG
gi|699977277|Isaria           CAAGGCAAAACAAATGAATCAAAACTTTCAACAACGGATCTCTTGGCTCTGGCATCGATG
gi|699210027|Beauveria      CAAGGCAAAACAAATGAATCAAAACTTTCAACAACGGATCTCTTGGCTCTGGCATCGATG
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gi|684907091|Beauveria      GACTACCCGCTGAACTTAAGCAT--------------------------------
gi|916346079|Beauveria      GACTACCCGCTGAACTTAAGCAT--------------------------------
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gi|699977277|Isaria GACTACCCGCTGAACTTAAGCAT----------

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gi|197365659|Beauveria ----------

NORMAL-BB AACTACTCCTCTATCCATGTCCTGG

gi|190335354|Beauveria ----------

gi|684907091|Beauveria ----------

gi|916346079|Beauveria ----------

gi|699977277|Isaria ----------

gi|699210027|Beauveria ----------

ITS 4 Oligonuclieotide primer and ITS 6 Oligonuclieotide primerSequence signals obtained and recognized by graphs. After obtaining sequence it analyzed on BLAST for identification of species. 99% sequences have obtained similar and 1% change has been reported.

1.Mutant *Beauveriabassiana* - UV light *Mutant Beauveria bassiana* have showed off white with colure and lightly less puffiness in physical characteristics. They want more germination time have required for obtaining growth on Petri plates. The image of *Mutant Beauveria bassiana* have showed bellow. Exposure of UV light on *Mutant Beauveria bassiana*, Transferred in fresh culture media, Incubation, Observation, Interpretation, DNA isolation, Quantification, PCR, and bioinformatical analyses have performed for both fungal species. All above methods have used for the identification of *Mutant Beauveria bassiana*

Fig. 4. Image of Mutant *Beauveria bassiana*

N- Cetyl- N, N, N-trimethyl- ammonium bromide (CTAB) method have followed for Total genomic DNA isolaton from the Mutant *Beauveria bassiana* fungi..

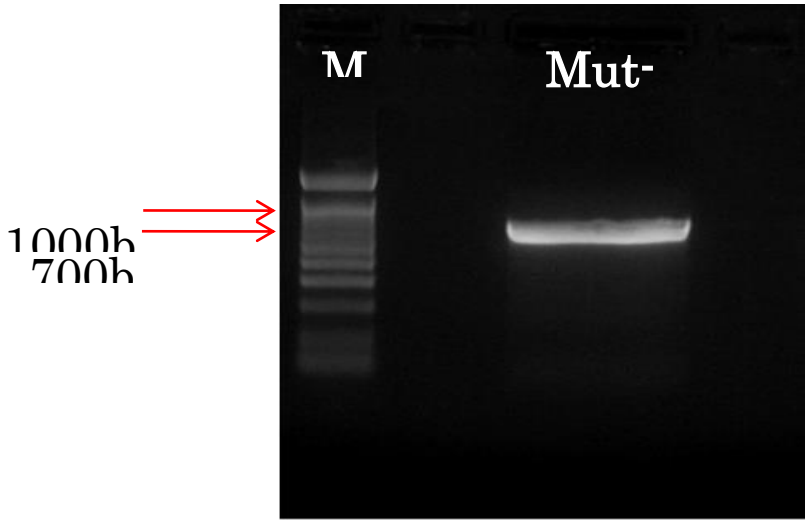

Fig. 5. Mutant *Beauveria bassiana* band obtained on electrophoresis gel

The quantity of the isolated DNA was checked in UV-VIS spectrophotometer (VivaspecBiophotometer, Germany). From the stock 1µl DNA was mixed with 49-µl sterile distilled water to get 50 times dilution. The A260/A280 ratio was recorded to check the purity of DNA preparation.Isolated DNA was amplified with the help of PCR techniques. PCR amplification of ITS region was done in 20 µl of reaction mixture containing PCR buffer, 1X (Kappa, SA); $MgCl_2$, 3 mM; dNTP mix, 0.25 mM; *Taq*DNA polymerase, 0.05 U; primer, 1 picomol and template DNA, 50 ng. Sterile nuclease free water is used as negative control.

Following CLUSTAL O (1.2.1) multiple sequence alignment obtained by next generator Sequencer. By this procedure obtained (588BP).

CGGCTGGTTCACCAACGGAGACCTTGTTACCTTGTTGGGGGACGCGGAGGGACATTACCGAGTTTTCAACTCCCTAACCCTTCT

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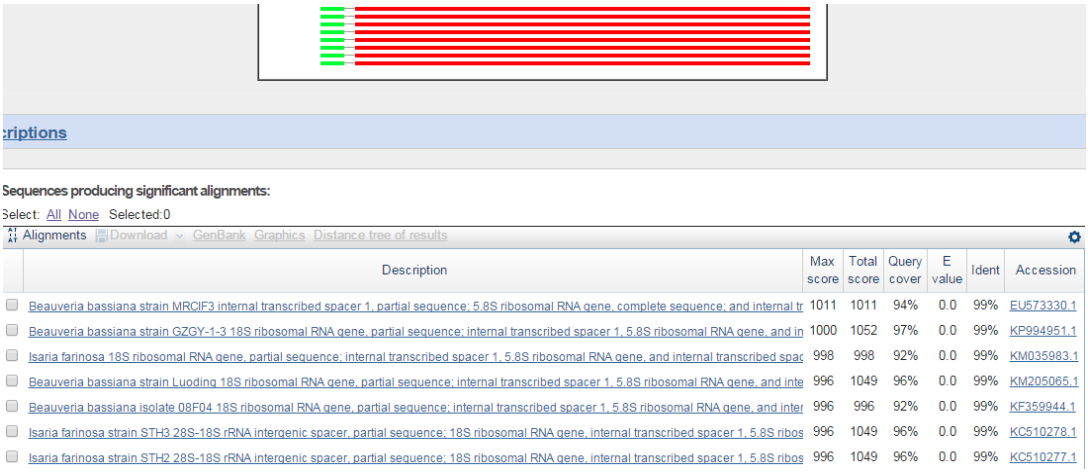

A

Fig. 6. CLUSTAL O (1.2.1) multiple sequence alignment

CLUSTAL O (1.2.1) multiple sequence alignment obtained.

gi|916346079|Beauveria ----------GCGGAGGGATCATTACCG

gi|699210027|Beauveria ----------GCGGAGGGATCATTACCG

gi|552339735|Beauveria ----------GCGGAGGGATCATTACCG

gi|476001871|Isaria ----------GCGGAGGGATCATTACCG

gi|190335354|Beauveria ----------CCTTTTTGGGGAAGGCGGAGGGACATTACCG

MUTANT-BB CGGCTGGTTCACCAACGGAGACCTTGTTACCTTGTTGGGGGACGCGGAGGGACATTACCG

gi|699977277|Isaria ----------

gi|916346079|Beauveria AGTTTTCAACTCCCTAACCCTTCTGTGAACCTACCTATCGTTGCTTCGGCGGACTCGCCC

gi|699210027|Beauveria AGTTTTCAACTCCCTAACCCTTCTGTGAACCTACCTATCGTTGCTTCGGCGGACTCGCCC

gi|552339735|Beauveria AGTTTTCAACTCCCTAACCCTTCTGTGAACCTACCTATCGTTGCTTCGGCGGACTCGCCC

gi|476001871|Isaria AGTTTTCAACTCCCTAACCCTTCTGTGAACCTACCTATCGTTGCTTCGGCGGACTCGCCC

gi|190335354|Beauveria AGTTTTCAACTCCCTAACCCTTCTGTGAACCTACCTATCGTTGCTTCGGCGGACTCGCCC

MUTANT-BB AGTTTTCAACTCCCTAACCCTTCTGTGAACCTACCTATCGTTGCTTCGGCGGACTCGCCC

gi|699977277|Isaria ----------

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gi|699210027|Beauveria CAGCCCGGACGCGGACTGGACCAGCGGCCCGCCGGGGACCTCAAACTCTTGTATTCCAGC

gi|552339735|Beauveria CAGCCCGGACGCGGACTGGACCAGCGGCCCGCCGGGGACCTCAAACTCTTGTATTCCAGC

gi|476001871|Isaria CAGCCCGGACGCGGACTGGACCAGCGGCCCGCCGGGGACCTCAAACTCTTGTATTCCAGC

gi|190335354|Beauveria CAGCCCGGACGCGGACTGGACCAGCGGCCCGCCGGGGACCTCAAACTCTTGTATTCCAGC

MUTANT-BB

CAGCCCGGACGCGGACTGGACCAGCGGCCCGCCGGGGACCTCAAACTCTTGTATTCCAGC

gi|699977277|Isaria - -GCCCGGACGCGGACTGGACCAGCGGCCCGCCGGGGACCTCAAACTCTTGTATTCCAGC

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            MUTANT-BB
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gi|699977277|Isaria         ATCTTCTGAATACGCCGCAAGGCAAAACAAATGAATCAAAACTTTCAACAACGGATCTCT
************************************************************
gi|916346079|Beauveria      TGGCTCTGGCATCGATGAAGAACGCAGCGAAACGCGATAAGTAATGTGAATTGCAGAATC
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gi|476001871|Isaria         TGGCTCTGGCATCGATGAAGAACGCAGCGAAACGCGATAAGTAATGTGAATTGCAGAATC
gi|190335354|Beauveria      TGGCTCTGGCATCGATGAAGAACGCAGCGAAACGCGATAAGTAATGTGAATTGCAGAATC
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gi|476001871|Isaria         CAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGCATTCTGGCGGGCATGCC
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gi|916346079|Beauveria      TGTTCGAGCGTCATTTCAACCCTCGACCTCCCCTTGGGGAGGTCGGCGTTGGGGACCGGC
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************************************************************
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                            ******************************************
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ITS 4 Oligonuclieotide primer and ITS 6 Oligonuclieotide primerSequence signals obtained and recognized by graphs. After obtaining sequence analyzed on BLAST for identification of species. After obtaining sequence it analyzed on BLAST for identification of species. 99% sequences have obtained similar 1% change has been occurred. The infection time duration have compared with normal *Beauveria bassiana* and mutant *Beauveria bassiana stain.*2×10^5 spore/ml, 2×10^7 spore/ml and 2×10^9 spore/ml counts have selected for detecting the morality ability of both wild type and mutant strain.

Spore counts of *Normal Beauveria bassiana* and *Mutant Beauveria bassiana*

Table.6. CFU counts of wild *Beauveria bassiana* and *Mutant Beauveria bassiana*

S.no.	CFU count of *Normal Beauveria bassiana*	CFU count of *Mutant Beauveria bassiana*

1.	2×10^5 spore/ml	2×10^5 spore/ml
2.	2×10^7 spore/ml	2×10^7 spore/ml
3.	2×10^9 spore/ml	2×10^9 spore/ml

Entomopathogenic fungus normal *Beauveria bassiana* & mutate *Beauveria bassiana* showed higher growth on potato dextrose agar broth. Shorter white mat type growth was up within 15 days & conidia were observed within 30 days. On the basis of obtained both strain sequence considered that mutation is occurred in new mutant strain. Among the Bentonite oil based liquid formulation were developed for both strain of *Beauveria bassiana* oil based highest efficacy with 2×10^5, 2×10^7 and 2×10^9 CFU spore/ml against larva of Helicoverpa armigera aphids and Thrips. A range of concentration 10-100% of each formulation was assay in laboratory & field trial. [5] *Beauveria bassiana* Bentonite oil base liquid formulation has been require one week for infection on larva of each. Significant control of mutant *Beauveria bassiana* showed by 2×10^9 spore per ml around 73-80% observed increasing dose of *Beauveria bassiana* showed failed to control of insect.

Table.7 *Mutant Beauveria bassiana* inhibition spectrum

S.no	**CFU count of Mutant *Beauveria bassiana***	**Helicoverpa armigera**	**Aphids**	**Thrips**
1.	2×10^5	40%	50%	45%
2.	2×10^7	80%	75%	78%
3.	2×10^9	64%	55%	50%

Table.8. Normal *Beauveria bassiana* inhibition spectrum

S.no	**CFU count of *Beauveria bassiana***	**Helicoverpa armigera**	**Aphids**	**Thrips**
1.	2×10^5	32%	40%	45%
2.	2×10^7	58%	50%	38%
3.	2×10^9	39%	47%	41%

Normal *Beauveria bassiana* form showed less control than mutant *Beauveria bassiana* strain. One studies so for suggested most economical dose of mutate *Beauveria bassiana*bentonite oil based liquid for as 2×10^7 spores/ml which showed 50% control against H.armizera, aphids & thrips. [6].

Bioassay in laboratory-

Bioassay fungal production *Beauveria bassiana* strain obtained against Helicoverpa armizera Aphids & Thrips were kept in growth chamber at optimum temperature & and humidity. Larva of aphids obtained from eggs & eat larva of soybean, toover and mung. 3 days old larva of each insect divided with mutant *Beauveria bassiana* formulation and examined the symbol of infection & motility of larva at each 12 hrs.

***Beauvera bassiana* Assay on pot**-: Mutant *Beauveria bassiana* formulation examined against larva of Helicoverpa armigera aphides & trips. *Beauveria bassiana* formulation spray leaves of soybean on plot & one pot kept as control. The larva applied on each pot 8-10 larvas each at different doses of formulation of Normal *Beauveria bassiana* & Mutant *Beauveria bassiana* larva allowed to stale down for 24 hrs. Water come on pot fungal infection examined in larva at weekly internal by experiment were repeated 5 time.In field experiment conclusively randomized block designed with each experimental plot measuring 2m×2m size, seeds of Soybean, Mung &Toover in 10×10 cm space in plot on all three crop agent trips, aphids, & Helicoverpa armizera on distinct dose formulated of Normal *Beauveria bassiana* & Mutant *Beauveria bassiana* were done at weekly interval for toxicity. Assay efficiency & potential of each dose of formulation were generated toxicity of larva were computed on the basis on the basis of critical difference. [7]

4.CONCLUSION

Beauveria bassiana bentonite oil based formulations can same as based spectrum biological control against Helicoverpa armigera, Aphids & Thrips in Soybean, Tover& Mung crop. Bentonite is best carrier material for *Beauveria bassiana* strain in then of economical cost in industries which maintain biological action for 2 year in bentonite oil based. Change in DNA sequence occurred by UV light with 90 minutes its confirmed with PCR and multiple sequenceing analysis. ITS 4 oligonucleotide primer and ITS 6 oligonucleotide primerSequence used for multiple sequencing. 18S ribosomal RNA gene used for identification of strain, sequence internal transcribed spacer 4 and Internal transcribed spacer 6 in Mutant *Beauveria bassiana* and Normal *Beauveria bassiana*. Obtained *Normal Beauveria bassiana* and mutant *Beauveria bassiana* strain are GZGY-1-3 and Tn-Bb-001 respectively, sequence against *Isaria farinose* strain and also with other which has mentioned in Fig.3 and 6. given in results. Significant control of mutant *Beauveria bassiana* showed by 2×10^9 spore per ml around 73-80% observed increasing dose of *Beauveria bassiana* showed failed to control of insect.

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me during the course of research.

CONFLICT OF INTEREST

The authors have no conflict of interest

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