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Research Article

IN VITRO STUDIES ON DIFFERENT MICROBIAL CONSORTIA FOR UTILIZATION OF CHLORMEQUAT CHLORIDE (CCC) AS FOOD SOURCE

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A B S T R A C T

Chlormequat Chloride (CCC) is a very stable compound used as PGR during foundation and forward pruning as growth retardant in vineyards. Because of non judicial use of CCC exceed the current MRLs at European level in the table grapes and *exports may reject fro*m India due to human health risks. So there is need for bioremediation to degrade CCC. Utilization abilities of three bacterial species consortia and three fungal species consortia to mineralize CCC at different time intervals were evaluated in this study developed from isolated pure microbes *BAA- *Azotobacter indicus* and *Acetobacter acetii*, *BBP- *Bacillus megaterium* and *Pseudomonas fluorescens,* ***BPB *Pseudomonas putida and Bacillus subtilis.* Three fungal species consortia *FAA- *Aspergillus nigar and Aspergillus awamorii,* **FTT-*Trichoderma viridi* and *Trichoderma harzianum,* ***FDC- *Trichoderma viridi and Trichoderma harzianum and Aspergillus nigar and Aspergillus awamori and Penicillium notatum* were mass multiplied, stabilized in liquid formulation *in vitro*. These microbial consortiums were screened for their capability of utilizing CCC as sole carbon energy source, and found it was rapidly utilized CCC beyond (500 $mg^{-1}L$) and showed abundance growth in a PDA and Nutrient Agar medium. The concentration of the CCC in the medium decreased exponentially with the exposure time. It is evident from the data that the maximum growth of bacterial consortium **BPB was observed in nutrient broth enriched with 2500 $mg^{-1}L$ and fungal consortium *** FDC in potato dextrose broth enriched with 1500 $mg^{-1}L$ CCC with residue 25.14 $mg^{-1}L$ and 24.60 $mg^{-1}L$ on 90th day reflecting 99.07 and 98.36 % utilization of CCC respectively, as a food source on LCMS-MS. Change in optical density, and spore biomass produced supported the biological transformation further resulting in mineralization of CCC.

INTRODUCTION

Health problems have become more and more complex during recent days. The increasing doses of insecticides, fungicides and other plant protection chemicals applied via foliar spray and drip are causes for newly emerging various types of human health diseases starting from newly born baby to elder persons also. All agrochemicals have their own half-life periods in soils, surface and in fruits (pulp), water table and in free environment residues. Amongst all crops grown major agrochemicals including Plant Growth Regulators (PGRs) use are on Cotton, grapes, vegetables, apples and pomegranates with floricultural crops grown in polyhouses have indiscriminate use of all types of agrochemicals to grow and protect crop from biotic, abiotic factors and to maximize the quality standards and yield. Chlormequat Chloride (CCC) is one of the most important PGR that reduces vegetative growth and influences the reproductive growth i.e. fruitfulness in grape vine and commercially more acceptable and commonly used. Present studies were made to see the impact of various microbial consortia on degradation of different concentrations, at different time intervals of CCC *Invitro* condition as food supplement. In vine yards during the Forward pruning (Fruit pruning) several biopesticides are used for the management of fungal diseases and insect pests during the growth stages of grape berries, out of which formulated microbes from *Trichoderma harzanium* genera, *Aspergillus* genera, *Penicilium* genera, *Pseudomonas* genera, *Bacillus* genera are very common and easily available on large scale. Based on this background information's the present work has been designed to search efficient bacterial and fungal consortia capable of degrading CCC in-vitro conditions with respect to the residues.

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MATERIALS AND METHODS

Plant growth regulator (PGR): Commercial grade Chlormequat Chloride (CCC) 50 % SL formulation was procured from local pesticide shop. Different required working concentrations as 500, 1000, 1500, 2000 and 2500 mg/l was

prepared with standard dilution method according to experiment design in D/W.

Pure Microbial Cultures: Isolated pure fungal microbes viz. *Trichoderma viride* NCIM No.1355 *Trichoderma harzianum* NCIM No.1373, *Aspergillus nigar* NCIM No.1213, *Aspergillus awamorii* NCIM No.1225, *Penicillium notatum* NCIM No.1206 and Bacterial cultures viz. *Azotobacter indicus* NCIM No.2055, *Acetobacter acetii* NCIM No.2116, *Bacillus megaterium* NCIM No.2475, *Pseudomonas fluorescens* NCIM No.3090, *Pseudomonas putida* NCIM No.2847, *Bacillus subtilis* NCIM No.5523 were obtained from National collection of Industrial Microorganisms-National chemical laboratory CSIR-NCIM-NCL Pune.

Chemicals and Reagents: PDA- Potato infusion 200 g, dextrose 20 g. Acetobacter aceti medium- Tryptone 10g, Yeast extract 10g, Glucose 10 g, Calcium Carbonate 10 g, pH 6.0. Burks medium K2HPO4 MgSO4 0.2 g, NaCl 0.2 g, FeSO4 0.001g, Mannitol 20 g pH 7.45-7.6. Bacillus megaterium medium- Sucrose 10g, K2HPO4 2.5g ,KH2PO4 2.5 g, DAP 1 g, MgSO4 0.2g, FeSO4 0.01g, MnSO4 0.007 g, Pseudomonas fluorescens medium- Protease peptone 20 g, K2HPO4 2 g, Glycerol 10g, MgSO4 2g, Nutrient broth - Beef extract 10 g, Nacl 5g, Peptone 10g, pH 7.0-7.5

Growth media Preparation: For different fungal cultures Potato Dextrose Broth (PDB), Nutrient broth and specific growth medium as per microbes was prepared, pH value was maintained by standard media preparation methods.

Maintenance of Fungal and Bacterial Cultures: Isolated pure fungal and bacterial microbes are stored at 4^0C, fungal microbes are sub-cultured and hyphae were grown separately on Potato Dextrose Agar and used for further studies. Bacterial microbes are grown on specific growth medium by streaking them on respective growth mediums in sterilized petriplates in a Laminar Air Flow chamber and incubated for 24 h at 37^0 C. The pure culture colonies were used for further studies. The cultures were maintained by regular subculturing at 15 days intervals.

Preparation of fungal microbe's inoculums*:* Potato dextrose broth medium was prepared in separate 250 ml conical flask, sterilized in autoclave at 121^0 C for 20 minutes, cooled at room temperature. Pre grown pure sub-cultured fungal hyphae transferred to individual conical flask separately under aseptic conditions (LAF) and kept at room temperature for incubation up to 72 h. mycelia growth, color change and sporulation was observed and recorded. These pure cultures are used as mother cultures for further studies.

Preparation of bacterial microbes inoculums: Individual mother culture of all bacterial microbes were prepared in respective selective mediums in separate 250 ml conical flasks, sterilized in autoclave at 121^0 C for 20 minutes cooled at room temperature. Pre grown pure sub-cultured bacterial microbes on nutrient Agar plates, single colony was transferred to individual conical flask with flame sterilized wire loop separately under aseptic conditions (LAF). The culture flasks were incubated on orbital shaker with 120 rpm at 30^0 C. These pure cultures are used as mother cultures for further studies.

Consortium preparation: Fungal and bacterial microbes were mass multiplied in sterilized conical flasks under aseptic and controlled conditions on specific growth mediums and stored. The microbial viability and other necessary biochemical tests are performed and recorded. On the basis of morphological parameter like Grams staining, motility, shape and biochemical characterizations type enzymes secretion, salt tolerance and utilization, compatibility among different microbes three bacterial consortium viz. *BAA- *Acetobactor aciti & Azotobactor indicus,* **BPB- *Pseudomonas putida & Bacillus subtilis*, *** BBP- *Bacillus megaterium & Pseudomonas fluorescens* were extensively grown on specific growth medium by combinations in equal mass and incubated overnight at room temperature on orbital shaker at 120 rpm. These consortiums are stored at room temperature and used for further studies as per protocol. Fungal consortium viz. *FTT- *Trichoderma viride* and *Trichoderma harzianum*, **FAA- *Aspergillus nigar* and *Aspergillus awamori*, ***FDC- *Trichoderma viridi* and *Trichoderma harzianum* and *Aspergillus nigar* and *Aspergillus awamori* and *Penicillium notatum* were prepared by growing them extensively on PDB by combinations in equal mass. These consortiums are stored at room temperature and used for further studies.

Enrichment of growth medium: Poison food technique was used for evaluating the impact of the CCC on the growth of antagonists. CCC was evaluated at 5 working concentrations. After cooling of the autoclaved PDB for the fungal microbial consortia * FAA, **FTT,***FDC, and bacterial consortia *BAA, **BPB, ***BBP - specific growth mediums were supplemented with 0.1, 0.2, 0.3, 0.4, 0.5 ml volume of commercial grade CCC with the help of micropipette to all respective growth mediums as 500, 1000, 1500, 2000, 2500 mg^{-1}L Chlormequat Chloride (CCC) initial concentration as source of food were incorporated aseptically into volume of respective growth mediums required for the growth of the consortia in separate surgical polyethylene tubes as per experimental protocols.

Inoculation of Consortia: The fermentation method has been adopted for the mass multiplication of microbes. 10 ml of prepared fungal and bacterial consortia were inoculated aseptically in respective growth mediums and adjusted the final volume to 100 ml with respective growth mediums as per experimental treatments to evaluate the impact of microbes on CCC degradation. Three replications are maintained for each treatment. Different mediums without consortium inoculums were kept as control. Tubes inoculated with fungal consortium are kept at room temperature. On completion of 30, 60 and 90 days fresh and dry mass was recorded. Tubes inoculated with bacterial consortium were kept on mechanical orbital shaker at 120 rpm for 12 h and incubated at 37 ^{0}C for 4 days in bacteriological incubator. Optical density was observed on Spectrophotometer at 560 nm and recorded for each treatment at 30, 60 & 90 days intervals and observations are recorded.

Quantification of CCC residue by LCMS-MS: Metabolites of Chlormequat Chloride (CCC) degradation by different fungal and bacterial consortium was analyzed by GCMS-MS at National referral laboratory, National Research Centre for grapes (NRL-NRCG) Pune after 30,60 and 90 days. Samples from each treatment were collected separately in a surgical urine bottles 25 ml each and stored at 4^0C temperature in refrigerator for 12 hrs. At NRL individual sample matrix fluid was centrifuged to get homogenized uniform matrix. The simple method was used for the extraction of CCC. 10 g of homogenized matrix was weighed in 50 ml plastic centrifuge tube. Then 20 ml of 0.1 % Formic acid in methanol was added, vortex for 5 min, then centrifuge at 10,000 rpm for 10 min.

Passed the supernatant through 0.2 µ filters and 2 ml supernatant was collected in 2 ml vial for residue analysis by LC/MS-MS. A residue of CCC was extracted from the homogeneous and representative sample with methanol after addition of isotope labeled standards. The quantity of CCC in the sample was calculated as the amount of chlormequat cations. The amount of CCC was expressed in $mg^{-1}L$.

RESULT AND DISCUSSION

Chlormequat Chloride (CCC) is a very stable compound used as PGR during foundation and forward pruning. Because of non judicial use of CCC which may be exceeding the current legal limit established at European level in the table grapes export from India. There is need for bioremediation to degrade CCC. Recent studies showed promising in PGPM research, one such technology is mixed inoculants consortium that interacts synergistically with each other which enhance the growth and protect from insects and disease causing pathogens (Esitken A, *et al*,.2010). Multiplication rate of bacterial microbes were evaluated in terms of mean optical density at 560 nm recorded and presented in table no. 1 maximum for bacterial consortium **BPB- 1.943, followed by *** BBP- 1.421 supplemented with 2500 mg/l CCC lowest growth recorded for *BAA- 0.983. *Pseudomonas putida* & *Bacillus subtilis* were extensively grown on specific growth medium combinations in equal mass supplemented with different CCC concentrations which was consumed intensively. Original color of commercial CCC start to disappeared with in 24 hrs and start to develop turbidity in growth medium treated with different bacterial consortia.

Several investigations are available for bioremediation of pesticides in soil, but for plant growth regulators no literature review available with reference to report on Enterobacter strain isolated from soil showed that the bacterium had strong phosphotriesterase activity and it hydrolyzed a 35 mg/l concentration of chlorpyrifos within 24h in liquid culture media (Singh *et al*., 2004). Investigation done by DeeAn Jones (1995) demonstrates that hydrolysis of the ester linkage in cypermethrin and the primary route of biodegradation. A novel study done by Maloney *et al*. (1988) also showed that microbial consortium can transform cypermethrin with a half-life of 7 to 14 days at a concentration of 50 mg/l in presence of tween 80. Bacillus cereus, Pseudomonas fluorescens and Achro-mobacter sp. were able to transform fenvalerate in presence of tween 80 within 5 days (Maloney *et al*.). Metabolic activities of bacteria, fungi and actinomycetes have the significant role in the degradation of pesticides. Biological methods are gaining interest due to their simplicity, high efficiency and cost effectiveness compared to other methods (Chandarn and Das, 2011).

Bioremediation is the use of microorganisms or their enzymes to break down and thereby detoxify dangerous chemicals in the environment (Obayori *et al*., 2009). The data presented in table no 2 revealed that minimum mean residues of CCC for all treatments on 90th day was 23.57 $mg^{-1}L$ for **BPB consortia followed by ***BBP- 27.64 $mg^{-1}L$ and *BAA-38.52 $mg^{-1}L$ was recorded.

The CCC utilization capacity of different bacterial consortiums as food supplements and the mean rate of degradation of

Table No 1 Impact on Optical density of bacterial consortia enriched with CCC

CCC	*BAA Consortia			**BPB Consortia			***BBP Consortia		
Dose mg/l	Day30	Day60	Day90	Day30	Day60	Day90	Day30	Day60	Day90
T1-500	0.245	0.261	0.286	0.721	0.853	0.898	0.456	0.478	0.498
T2-1000	0.376	0.423	0.445	0.986	1.009	1.249	0.567	0.603	0.793
T3-1500	0.398	0.436	0.465	1.154	1.243	1.356	0.789	0.923	1.109
T4-2000	0.732	0.762	0.798	1.256	1.475	1.762	0.963	1.009	1.346
T5-2500	0.921	0.967	0.983	1.388	1.634	1.943	1.103	1.302	1.421
Control	0.221	0.245	0.294	0.456	0.469	0.497	0.321	0.351	0.387
Mean	0.482	0.515	0.545	0.993	1.113	1.284	0.699	0.777	0.925
SEM±	0.115	0.117	0.114	0.143	0.174	0.218	0.123	0.147	0.179
CD@5%	0.024	0.025	0.027	0.049	0.055	0.064	0.034	0.038	0.046

Consortium *BAA- *Acetobactor aciti & Azotobactor indicus,* **BPB- *Pseudomonas putida & Bacillus subtilis*, *** BBP- *Bacillus megaterium & Pseudomonas fluorescens*

Table No 2 Impact of Bacterial consortia on CCC residue (in mg/ $mg^{-1}L$)

CCC	*BAA Consortia			**BPB Consortia			***BBP Consortia		
Dose ppm	Day30	Day60	Day90	Day30	Day60	Day90	Day30	Day60	Day90
T1-500	56.23	25.75	23.12	50.70	18.40	9.70	48.20	19.32	11.23
T1-Control	63.10	31.50	18.23	63.10	31.50	18.23	63.10	31.50	18.23
T2-1000	125.33	33.54	29.45	115	25.80	22.60	118.42	29.54	24.17
T2-Control	192.00	86.30	44.76	192.00	86.30	44.76	192.00	86.30	44.76
T3-1500	187.12	46.30	32.48	168.00	31.50	28.35	167.30	43.23	33.56
T3-Control	194.00	78.40	35.30	194.00	78.40	35.30	194.00	78.40	35.30
T4-2000	256.50	65.89	51.23	225.00	36.40	32.10	236.00	45.78	39.51
T4-Control	289.00	156.80	82.32	289.00	156.80	82.32	289.00	156.80	82.32
T5-2500	287.46	78.12	56.32	253.00	41.30	25.14	265.23	48.43	29.76
T5-Control	292.00	165.20	85.80	292.00	165.20	85.80	292.00	165.20	85.80
Abs. Control	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
Mean (T)	182.52	49.92	**38.52**	162.94	30.68	**23.57**	167.07	37.26	**27.64**
Mean (C)	206.02	103.64	53.28	206.02	103.64	53.28	206.02	103.64	53.28
SE(m) ±	1.847	0.258	0.130	1.778	0.204	0.181	1.941	0.205	0.182
C.D. at 5%	6.118	0.855	0.431	5.888	0.675	0.600	6.428	0.680	0.603

Control- with respective $mg^{-1}L$ CCC without microbial consortia, Abs Control- Microbial consortia without CCC, Consortium Consortium *BAA- *Acetobactor aciti & Azotobactor indicus,* **BPB- *Pseudomonas putida & Bacillus subtilis*, *** BBP- *Bacillus megaterium & Pseudomonas fluorescens*

different concentrations of CCC at 30, 60 and 90 days in terms of mg/kg recorded for bacterial consortium **BPB with maximum utilization 72.19 $mg^{-1}L$ followed by ***BBP- 77.31 $mg^{-1}L$ and *BAA- 90.31 $mg^{-1}L$ as compared to control 120.98 $mg^{-1}L$.

Chaussonnerie *et al*. (2015) isolated and characterized two closely related species of Citrobacter amalonaticus those were able to transform recalcitrant chlordecone from pesticide contaminated soil. Endosulfan degradation by P. aeruginosa G1 (88.5%), Stenotrophomonas maltophilia G2 (85.5%), B. atrophaeus G3 (64.4%), Citrobacter amolonaticus G4 (56.7%) and Acinetobacter lowffii G5 (80.2%) was reported (Ozdal *et al*., 2016).

Abundance and distribution of potential microbes and functional genes associated with pentachlorophenol anaerobic mineralization in a continuous flow reactor was studied by Li *et al*.2016). Microbes with potential reductive dechlorinators; Dehalobacter, Sulfospirillum, Deslfitobacterium and Desulfovibrio spp. and phenol degrader Cryptanerobacter and Syntrophus spp. In case of all fungal consortia within 48 hrs after inoculation of consortium dense clouds of mycelia were found in all treatments and replicates and mycelium growth with dense sporulation was observed after 30 days with increased fresh and dry weight.

Table No 3 Impact on fungal consortia fresh biomass (in g) enriched with CCC

CCC	*FAA Consortia (F)			**FTT Consortia (F)			***FDC Consortia (F)		
Dose mg/l	Day30	Day60	Day90	Day30	Day60	Day90	Day30	Day60	Day90
T1-500	4.58	4.80	4.80	4.53	4.90	5.13	5.53	5.80	6.32
T2-1000	5.30	5.70	5.75	4.83	5.10	5.30	5.90	6.30	6.85
T3-1500	6.13	6.30	6.55	5.16	5.30	5.42	6.32	6.70	6.92
T4-2000	6.45	6.70	6.90	5.43	5.60	5.85	6.48	6.90	7.03
T5-2500	7.96	8.45	9.16	6.48	7.98	5.93	7.38	8.14	9.76
Control	4.23	4.50	4.70	4.03	4.60	4.90	4.91	5.30	5.80
SE(m) ±	0.023	0.025	0.029	0.014	0.021	0.008	0.014	0.017	0.024
C.D. at 5%	0.073	0.079	0.091	0.044	0.067	0.025	0.043	0.053	0.076

Consortium *FAA-*Aspergillus nigar & Aspergillus awamori,* **FTT- *Trichoderma viridi & Trichoderma harzianum,* *** FDC- *Aspergillus nigar, Aspergillus awamori, Trichoderma viridi, Trichoderna harzianum, Penicillium notatum.* F- Fresh weight.

Table No 4 Impact on fungal consortia dry biomass (in g) enriched with CCC

CCC	*FAA Consortia (D)			**FTT Consortia (D)			***FDC Consortia (D)		
Dose mg/l	Day30	Day60	Day90	Day30	Day60	Day90	Day30	Day60	Day90
T1-500	1.13	1.10	1.47	0.98	1.21	1.52	1.43	1.53	1.68
T2-1000	1.43	1.74	1.79	1.23	1.31	1.69	1.86	1.98	2.03
T3-1500	2.35	2.14	2.86	1.43	1.53	1.73	1.98	2.12	2.45
T4-2000	2.73	2.98	3.01	1.55	1.68	1.76	1.98	2.16	3.20
T5-2500	3.40	3.98	4.10	2.14	2.67	3.13	2.30	2.76	3.37
Control	0.78	1.10	1.34	0.78	1.15	1.34	1.21	1.43	1.52
SE(m) ±	0.018	0.020	0.020	0.008	0.010	0.012	0.007	0.008	0.013
C.D. at 5%	0.058	0.063	0.065	0.026	0.032	0.040	0.023	0.024	0.043

Consortium *FAA-*Aspergillus nigar & Aspergillus awamori,* **FTT- *Trichoderma viridi & Trichoderma harzianum,* *** FDC- *Aspergillus nigar, Aspergillus awamori, Trichoderma viridi, Trichoderna harzianum, Penicillium notatum.* D- Dry weight.

Table No 5 Impact of fungal consortia on CCC degradation in $mg^{-1}L$

CCC	*FAA Consortia (F)			**FTT Consortia (F)			***FDC Consortia (F)		
Dose mg/l	Day30	Day60	Day90	Day30	Day60	Day90	Day30	Day60	Day90
T1-500	52.10	19.40	7.80	58.10	26.40	10.50	35.80	7.98	4.24
T1-Control	63.10	31.50	18.23	63.10	31.50	18.23	63.10	31.50	18.23
T2-1000	114.00	25.90	23.54	128.00	25.20	22.10	103.00	24.10	10.40
T2-Control	192.00	86.30	44.76	192.00	86.30	44.76	192.00	86.30	44.76
T3-1500	162.00	30.40	26.80	140.00	30.30	28.60	160.00	27.80	24.60
T3-Control	194.00	78.40	35.30	194.00	78.40	35.30	194.00	78.40	35.30
T4-2000	214.00	34.10	32.30	250.00	34.50	31.10	211.00	33.30	30.80
T4-Control	289.00	156.80	82.32	289.00	156.80	82.32	289.00	156.80	82.32
T5-2500	245.00	37.30	33.80	253.00	36.70	33.40	261.00	35.40	33.10
T5-Control	292.00	165.20	85.80	292.00	165.20	85.80	292.00	165.20	85.80
Abs. Control	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
Mean (T)	157.42	29.42	24.84	165.82	30.6	25.14	154.16	25.71	20.62
Mean (C)	206.02	103.64	53.28	206.02	103.64	53.28	206.02	103.64	53.28
SE(m) ±	1.965	0.316	0.227	1.806	0.223	0.199	1.833	0.269	0.144
C.D. at 5%	6.509	1.048	0.751	5.982	0.739	0.660	6.607	0.892	0.478

Control- with respective $mg^{-1}L$ CCC without microbial consortia, Abs Control- Microbial consortia without CCC, Consortium *FAA-*Aspergillus nigar & Aspergillus awamori,* **FTT- *Trichoderma viride & Trichoderma harzianum,* *** FDC- *Aspergillus nigar, Aspergillus awamorii, Trichoderma viride, Trichoderna harzianum, Penicillium notatum.*

In this study microbial degradation activity was found to be enhanced after 60 day incubation. The biochar amended soil contained Chryseobacterium, Flavobacterium, Dadobacterium, and Pseudomonadaceae members. Increased microbiological dehydrogenase activity due to biochar amendment was responsible for enhanced degradation of organochlorine that was otherwise attenuated due to arsenic contamination (Gregory *et al*., 2015).

The isolated pure fungal cultures were extensively grown on specific growth medium- PDB supplemented with different CCC concentrations which was consumed intensively.

The data presented in table no.3&4 revealed that maximum mycelia fresh biomass 9.76 g, dry biomass 3.37 g obtained for fungal consortium ***FDC, followed by **FAA- 9.16 g, dry biomass 4.10 g and minimum for *FTT 5.93 g, dry biomass

3.13 g in PDB per 100 ml supplemented with 2500 $mg^{-1}L$ CCC on 90th day after inoculation. The data presented in table no 5 revealed that minimum mean residues of CCC for all treatments on 90th day was 20.62 $mg^{-1}L$ for ***FDC consortia followed by *FAA- 24.84 $mg^{-1}L$ and **FTT-25.14 $mg^{-1}L$ was recorded. The CCC utilization capacity of different fungal consortiums as food supplements and the mean rate of degradation of different concentrations of CCC at 30, 60 and 90 days in terms of $mg^{-1}L$ recorded for fungal consortium ***FDC with maximum utilization 66.83 $mg^{-1}L$ followed by *FAA- 70.56 $mg^{-1}L$ and **FTT- 73.85 $mg^{-1}L$ as compared to control 120.98 $mg^{-1}L$. Our goal in this study was to identify the most prominent fungal and bacterial strain to utilize the Chlormequat chloride as a food source and produce maximum biomass, for future research study regarding the mineralization of CCC residues, used on grape vine.

CONCLUSION

At present Plant Growth Regulators are extensively used by grape growers on the all varieties of vine. Due to indiscriminate and uncontrolled use of PGRs per application leads to persistence and accumulated in or on grape berries which contaminate the edible part and cause health hazards. Degradation is breakdown of a complex substrate into simple product leading to mineralization. According to Gales (1952) principal of microbial infallibility, for every naturally occurring organic compound there is a microbe enzyme system capable of its degradation. In the present study microbial consortiums were screened for their capability of utilizing CCC as sole carbon energy source, and found it was rapidly utilized CCC beyond (500 $mg^{-1}L$) and showed abundance growth in a PDA and Nutrient Agar medium. The concentration of the CCC in the medium decreased exponentially with the exposure time. It is evident from the data that the maximum growth of bacterial consortium **BPB was observed in nutrient broth enriched with 2500 $mg^{-1}L$ and fungal consortium *** FDC in potato dextrose broth enriched with 1500 $mg^{-1}L$ CCC with residue 25.14 $mg^{-1}L$ and 24.60 $mg^{-1}L$ on 90th day reflecting 99.07 and 98.36 % utilization of CCC respectively, as a food source on LCMS-MS. Change in optical density, and spore biomass produced supported the biological transformation further resulting in mineralization of CCC. Further investigations are going on at National Research Centre for Grapes Pune, such as degradation enzymes secreted and other biochemical aspects in case of Chlormequat Chloride concerning with food safety in grapes.

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