Noteworthy Bio Resource Utilization and Eco-Friendly Bioconversion Approaches of Versatile Invasive Aquatic Weed Water Hyacinth (*Eichhornia Crassipes*) Biomass - A Green Strategy for Aquatic Environmental Safety and Management of Aquatic Weeds.

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Abstract

Eichhornia crassipes (Water hyacinth) is considered as a hazardous weed in numerous places of the world. Despite of its harmful effects, the weed offers potential substrate for production of monetarily industrial important and value added products. In the present study, an eco-friendly bioconversion or biotransformation of processed water hyacinth biomass (WHB) into high efficiency fuel, adsorbent for brewery industry waste water treatment coupled methylene blue dye, biofertilizer and nutrient media for viable inoculam production of fungal biopesticide *Nomuraea rileyi* was carried out adopting green science in situ principles. Bioconversion of the WHB was initiated by alkali treatment which brought about notable differences in physical texture and chemical composition. Pre-treated WHB was enzymatically hydrolysed into soluble simple sugars followed by fermentation of the sugar into ethanol with 65.2 % total yield with 77.3 g/l final concentration. Batch studies reveals that WHB brought about effective reduction of various physicio chemical properties and notable adsorption efficacy. Plant growth promoting effect was studied using green gram (*Vigna mungo*). Green gram grew on soil under pot culture technique inoculated with WHB uncovers imperative plant growth promotion effect. Culture medium prepared from reconstituted WHB supported viable inoculum production of potential fungal biopesticide *Beauveria bassiana*. Fungal inoculum derived from the WHB based culture medium was effective against larval instars of castor semi-looper *Achaea janata* in terms of high mortality.The present examination uncovers the conceivable viable bioconversion of water hyacinth biomass (WHB) into different worth included vitality, ecological as gives a green procedure to cleaner production and the executives of aquatic invasive weeds.

Introduction

Pollution of water ecosystem by aquatic biota, mainly aquatic invasive weeds, records several ecological and economic issues in various parts of the world (Anudeep et al. 2014. Baruah et al 2018).Aquatic weeds, a significant type of biomass composed of micro, macroalgae, macrophytes that grow in water bodies and cause undesirable effect on the aquatic life and water quality (Hebes et al. 2018). Due to their fast growth rate, diverse propagation types, and ubiquitous distribution are considered serious global ecological problems (Kaur et al. 2017). Aquatic weeds are also known to cause a notable effect on local marine conditions, mainly the temperature of the water, the chemical composition of sediment, and the nutritional cycling phenomenon, all of which cause severe ecological damage to aquatic life (Ribaudo et al. 2018). Recent reports also reveal that aquatic weeds cause a marked effect on the marine ecosystem's various parameters like hydrological, socio-economic and ecological activities (Clement Nyamekye et al. 2021).

Ignoring the debilitating effects of aquatic weeds, we receive some specific advantages based on biomass because of their chemical composition, mainly fermentable simple sugars which can be used as the substrate for various economic important or value-added products (Mohamed et al 2019, Alam et al. 2021). Obruka et al (2015) reveal the potential use of aquatic plant biomass for different applications like feedstocks for biofuels, bio fertilisers and phytoremediation agents. The advantages of aquatic weeds

biomass usage like the absence of negative interaction like completion with farmland economic important crops, low cost and remediation of toxic organic compounds including heavy metals have gained more attention in the biotechnological utilisation and green weed control or management strategy (Illo et al 2020).

Among the diverse aquatic weeds, water hyacinth (*Eichhornia crassipes*) is an important invasive perennial weed associated with various marine systems of tropical, subtropical areas. Deterioration of the aquatic ecosystem by this invasive weed is massive due to higher growth rate, depletion of oxygen, reduction of light penetration which all prevents marine fauna and flora life (Zhang et al. 2018). Though the harmful impact of water hyacinth has been studied elaborately, some recent studies revealed the potential application of water hyacinth biomass (WHB). The lower amount of lignin and the high cellulose content that can be easily converted into simple sugar residues of the biomass attracts the scientific community for the large-scale utilisation of processed biomass as a versatile substrate for producing various value-added products. An extensive review by Sindhu et al. 2017 revealed the potential use of WHB as a source to produce important industrial polymers, solvents, biofuels, biofertilisers, biogas, animal feeds. Specific physicochemical properties of water hyacinth biomass can be utilised to produce biofuels (bioethanol) and biogas (Bote et al. 2020). A review by Illo et al. 2020 also showed the utilisation of WHB as a potential source for a wide range of industrially important products. The biological activities of cellulose composite prepared from the processed biomass of WH fabricated with pesticide and curcumin have recently been studied by Avinash et al (2020). Briquettes have been prepared from the WHB by Munjeri et al (2016) can be used as a thermal energy source, particularly for communities staying in the vicinity of the affected water source

With this background, the current study is undertaken to evaluate the bioresource utilisation approaches. of WHB via green science principles for diverse biotechnological applications. Alkali treated WHB was converted into a soluble, fermented sugar-rich substrate by cellulolytic organisms followed by bioethanol fermentation by yeast strain. Plant growth-promoting efficacy of processed biomass alone and its synergistic effect with bacterial bio fertilisers was checked with *Vigna mungo* under pot conditions. Effect of WHB based culture media on the *in vitro* multiplication of potential fungal biopesticide *Beauveria bassiana*. Wastewater treatment efficacy coupled methylene blue dye adsorption was also checked under batch conditions. We hope that this study will give insight into the management of aquatic weeds with biotechnology principles that may pave the way for a system to affect invasive weeds effectively.

Materials And Methods

Enrichment of composition of collected water hyacinth biomass

Water hyacinth samples were collected from the lake of Sholinganallur 12.9010 ^{o} N,80.2279^{o}E Chennai, Tamil Nadu, India (Fig. 1a,b,c). Pre-treatment of the collected biomass was done by the soluble alkali. Leaves were expelled from the stalk and the gathered leaf material was washed with tap water followed by distilled water. Washed material was slashed into littler pieces, soaked in known volume of sodium

hydroxide solution (1.5 %) for 48 hours. Following 48 hours of alkali treatment, the drenched pieces were dried in a hot air oven for over night to expel the dampness content. The dried material was finely powdered utilizing residential blend (Fig. 1d, e). Homogenized material was sieved through 22 work and the sieved powder was stored in separate container, utilized for further studies. Scanning electron microscopy (SEM) studies was carried out to determine structural changes of WHB before and after alkali treatment. SEM micrographs were taken to determine the structural changes.

Ethanol production from the processed biomass

In the case of bioethanol production, solid state fermentation (SSF) was adopted for the preparation of hydrolysate- which consists of digested simple sugar from the biomass treated with cellulolytic fungal co culture. Hydrolysate thus obtained was allowed to fermentation under batch condition using yeast *Saccharomyces cerevisiae*. Total yield and concentration of final fermented bioethanol was measured.

Hydrolysate preparation

Solid state fermentation was adopted for the hydrolysate preparation from the alkali treated biomass. Processed biomass (60 g) was transferred to 250 ml of conical flasks, followed by sterilization using autoclave. After sterilization, the moisture content of the substrate was adjusted to 0, 1, 2.5, 5.0 7.5 &10.0 % using sterile distilled water under aseptic condition. Cellulolytic fungal strains used in the present study, *Trichoderma* sp and *Aspergillus niger* were isolated from agriculture field soil samples. Isolated fungal strains were identified based on morphological and cultural characteristics on solid media (Fig. 3).Screening of cellulolytic activity of the fungal strains was done by the method of Vyas & Vyas (2005).Pure culture of the respective fungal strain was maintained on potato dextrose agar (PDA) slant. 1.0% (w/v) of co culture of cellulolytic fungal *(Trichoderma* sp and *Aspergillus niger*) inoculum were inoculated into the conical flasks respectively. Inocula of the respective fungal strain was prepared in mineral broth medium supplemented with substrate solution (0.1 % carboxyl methyl cellulose). Seeded flasks were incubated for five days at 35^{o}C under static condition. After the incubation period, the respective flask was flooded with sterile distilled water, kept, under shaking condition at ambient temperature for one hour. Content was filtered through the whatman No 1 filter paper, the collected filtrate was centrifuged at 10,000 rpm. Supernatant thus obtained was used as the source of hydrolysate for bioethanol production.The concentration of total reducing sugars was estimated using the DNS method (Miller 1959).

Conversion of biomass derived hydrolysate into ethanol by yeast mediated fermentation

Batch fermentation study was adopted for ethanol production using yeast strain. Known volume of filter sterilized hydrolysate thus obtained was inoculated to the sterile basal medium (Mukhopadhya et al 2008).Two ml of *Saccharomyces cerevisiae* inoculum was added to the media. For inocula preparation, a loopful of *S.cerevisiae* culture (obtained from Microbial Type culture collection- MTCC ,Chandigurh ,India) was inoculated into sabouraud dextrose broth. Inoculated broth was incubated at 35°C for 48 hours

under shaking condition. After the incubation period, the fermented broth was filtered,collected filtrate was analysed for total ethanol content (AOAC 1990).

In this study, methylene blue was selected for the adsorption efficacy of WHB under batch condition. Methylene blue analytical grade (Qualigens) was prepared as required concentration (100,125,150,175 and 200 ppm) in 100 ml of distilled water followed by the addition of 0.1 and 1.0 % dosage of WHB separately. Seeded flasks were incubated under orbital shaker (100 rpm) at ambient temperature. At the respective time periods, the samples withdrawn from the flasks and the amount of residual dye was m0easured at 668 nm using Shimadzu-1800 spectrophotometer.Effect of WHB on the chemical oxygen demand (COD) of the methylene blue solution was carried out.

Water treatment efficacy

Effect of WHB on the waste water treatment efficacy was studied by batch studies under controlled condition. Brewery industry effluent with the following parameters pH 9.4,1860 mg/L TDS,980 mg/L BOD,1875 mg /L COD, 45.3 mg/L TOC,32.4 mg/L TN,36.4 mg/L TP) that collected from Empee brewery industry, Padappai, Kanchipuram, Tamil Nadu was used in this study to check the water treatment efficacy. Known quantity of collected sample (1L) was transferred to 2L of conical flask followed by the addition of 1.0 % WHB. Seeded flasks were incubated at ambient temperature under shaking condition (150 RPM). To determine the WHB mediated waste water treatment efficacy, changes in the various chemical parameters (pH ,total dissolved solids (TDS),chemical oxygen demand (COD),biological oxygen demand (BOD),total organic carbom, total nitrogen, total phosphorous) was done by using standard methods of APHA. Biofilm inhibition efficacy was also investigated by modified method of Toole & Kolter, 1998). In this method, known volume of collected water sample (5ml) was transferred to the sterile screw cap vial under aseptic condition followed by incubation without shaking at 37°C for three days. After the incubation period, adherent biofilm was stained by crystal violet followed by solubilisation with ethanol. Biofilm forming efficacy was calculated by measuring the optical density of the ethanol solubilized biofilm at 570 nm. Morphological changes of the biofilm after exposure to the WHB treatment was studied by scanning electron microscopy analysis (Namasivayam et al 2019). Adherent biofilm was isolated from the inoculated vial, fixed with glutaraldehyde and observed for morphological changes.

Evaluation of reusability of WHB treated water and dye

Reusability of WHB treated brewery effluent and methylene blue was done by phytotoxicity assessment. Phytotoxicity assessment was studied by determination of germination index (seedlings emergence percentage).

Seeds collection and treatment

Healthy seeds of *Vigna mungo* were collected from seeds collection centre of Chengalpattu Agriculture Department, Washing of the seeds with the sterile distilled water was done before seed treatment. Washed seeds were grouped into control and treatment followed by soaking in the suspension derived

from the respective treatment at room temperature for 1 hour. After the incubation period, treated seeds from the respective treatment groups were transferred to the sterile petri plate, incubated at ambient condition. From the data of seedlings emergence in the respective group, germination index was determined.

Zebra fish developmental toxicity

Non target toxic effect was studied by determination of developmental parameters of zebra fish as described in our recent study (Namasivayam et al 2018).

Evaluation of enhanced growth stimulation efficacy (GSE)

Enhancement of growth stimulation efficacy (GSE) of biomass and its synergistic impact with different biofertilizers *Rhizobium* sp, *Azospirillum* sp and *Phosphobacterium* sp on *V.mungo* was examined on green gram (Vigna mongo) (under pot culture condition. Particular biofertilizer was utilized as powder definition. One gram of powder detailing of individual biofertilizer was blended well in with one gram of WHB independently and moved to the mud pot pre-loaded up with soil (2 Kg/pot).Three replications and control was kept up for every treatment. Healthy seeds of *V.mungo* were moved to the pots and permitted to develop Water was applied every day. Plant growth advancing impact was studied by estimating different parameters of tested plants after 30 and 60 days treatment.s

Influence of WHB media on biomass production of fungal biopesticidal agent *Beauveria bassiana*

Assessment of WHB on the biomass and spore production of potential fungal biopesticide *Beauveria bassiana* was done under *in vitro* condition and the pesticidal activity of biomass in this manner acquired from WHB medium was screened against castor caterpillar *Achaea janata*.

Isolation of *B.bassiana*

The tested fungal strain was isolated from groundnut nearby field soil tests embracing soil dilution technique as portrayed by our recent reports (Namasivayam et al 2018). Well homogenized collected soil sample was serially diluted and the aliquots was spread plated on selective media and the isolated fungal culture was distinguished dependent on the morphological and cultural characteristics embracing standard strategies. Isolated pure culture was maintained on agar slant. Inoculum of the fungal strain for mass production was derived from agar slant.

Evaluation of biomass and spore production of fungal biopsticidal agent *Beauveria bassiana*

WHB based liquor medium was prepared by suspending different concentration of homogenized WHB (0.1%, 0.25%, 0.5%, 1%, 2%, 2.5%, 5%) in 100 mL of distilled water, sterilized by autoclaving. After sterilization, 0.1 ml of the inoculum was inoculated into the WHB medium. Seeded flasks were incubated under static condition at 28ºC for 5 days. After the incubation time, the medium was filtered and the dry weight of collected biomass was recorded. For the determination of spore count, collected biomass was

washed with sterile distilled water,centrifuged at 2,500 rpm for 10 minutes. Collected supernatant was used as the source of inoculum.

The impact of water hyacinth media on spore germination was additionally contemplated utilizing cavity slide method. Spore suspension thus obtained was transferred to the sterile cavity slide kept in the petriplates fixed with sterile filter papers moistened with sterile distilled water, incubated at 28ºC. Spore germination was observed under microscope and the information got was utilized to record germination index (%)..

Effect of fungal spores on survival parameters of *Achaea janata*

Assessment of the pesticidal action of spores got from the fungal biomass was tried against significant castor semi-looper *Achaea janata*. The pesticidal action was confirmed by examining total survival rate of respective treatment groups

Effect of fungal spores on cumulative mortality against *Achaea janata*

Various larval instars of *Achaea janata* (1-1V) were used to determine the insecticidal activity of *B.bassiana*. Spore suspension was gotten from the fungal strain was utilized as the wellspring of inoculum for the pesticidal activity Spore concentration of the suspension was previously determined and aliquots were set up from the stock utilized for additional investigations. In this study, respective larval instar collected from the laboratory stock culture were sprayed with various aliquots of spore suspension using ultra-low volume sprayer. Triplicates and control was maintained. Everyday observation on larval mortality was recorded for a time of 4 days. Total mortality was recorded in the individual measurements of treatment which was used to predict lethal concentration 50 (Finney, 1962).

Results And Discussion

Waste biomass lignocellulosic plants materials primarily composed of cellulose, lignin have a great potential for the production of a wide range of value added products (Obruca et al 2015). Various reports reveal the possible utilization of pre-treated lignocellulosic biomass for the diverse biotechnological applications. In this study an attempt is made to constructively incorporate the field of biotechnology in resource study by harnessing the integral advantage of the biotechnology principles with significant resource strategies. Eco friendly bio conversion methods were adopted for the diverse biotechnological applications of WHB through green science principles studied in this present investigation would suggest the possible utilization of lignocellulosic waste biomass as a potential source for the value added products and energy material.

Effect of pre-treatment of WHB on the chemical composition

Soluble alkali treatment of WHB with NaOH (2%) was initially carried out. Pre- treatment is essential to reduce the sugar loss, facilitates the enzymatic hydrolysis, and prevents the accumulation of fermentation inhibitors (Nishu 2018).Alkali treatment reveals notable changes in the chemical

composition which can be easily inferred from Fig. 2. Prior to pre-treatment with soluble base, WHB recorded cellulose 29.2 %, hemicellulose 33.4 % ,lignin 4.0 %, absolute nitrogen 2.3 %. Soluble base treated WHB shows eminent contrasts in substance organization. Cellulose, hemicellulose and rough nitrogen content were seen as expanded in soluble base treated WHB which recorded 32.1% cellulose, 35% hemicellulose, 0.6 % lignin, 4.3% complete nitrogen. Critical decrease of lignin was seen in soluble alkali treated WHB which yielded 0.6 %. Pre-treatment ordinarily separates the naturally visible inflexibility of the biomass and lessens the physical hindrances to mass vehicle (Liu et al. 2009). Fig. 2b portrays the SEM micrograph of both control and antacid treated WHB which shows the adjustments in the inflexibility of soluble base treated WHB or basic trustworthiness misfortune. This examination obviously uncovers that alkali pre-treatment achieved bioconversion of WHB complex polysaccharides into basic sugars which can be used for different bio asset techniques.

Effect of biomass on ethanol production

Ethanol was produced from WHB determined hydrolysate by enzymatic hydrolysis of pre-treated WHB utilizing cellulolytic fungal strains *Trichoderma* sp and *Aspergillus* sp. Both the strains were hydrolysed complex cellulose substance of WHB into simple sugars rich hydrolysate which could be utilized as the media for ethanol fermentation.

Hydrolysate –medium for fermentation preparation

Hydrolysate –medium for ethanol production was prepared from processed biomass treated with cellulolytic fungal organisms. Respective fungal strain was distinguished by morphological characters like culture morphology, infinitesimal assessment of spores utilizing lactophenol cotton blue staining (Humber 1997). On PDA media, *Trichoderma* framed green conidia and scattered all in all plate (Fig. 3a). Microscopic assessment of conidia with lactophenol cotton blue uncovered 2.5 micron green globose molded. *Aspergillus* sp framed thick dark elevated mycelia on PDA (Fig. 3b).Lactophenol cotton blue staining shows dull darker globose conidia.

Cellulolytic fungal strains mediated hydrolysate thus obtained was analysed for soluble sugars which could be utilized as the substrate for ethanol fermentation. Hydrolysate was obtained from the biomass treated with cellulolytic fungal strain adopting solid st ate fermentation strategy. Hydrolysate thus obtained was estimated for glucose and reducing sugars. Results clearly demonstrates that the yield of reducing sugars and glucose expanded continuously alongside the expanded brooding time and dampness content. A progressive increment of glucose and reducing sugars was seen in expanding convergence of moisture content. Be that as it may, prominent decrease on the yield of both glucose and reducing sugars was seen in 10.0 % moisture content. Impact of incubation time on the yield of reducing sugars and glucose at the respective moisture content treatment was also examined. Fig. 4a, b shows cellulolytic bacterial and fungal hydrolysis yield of reducing sugars and glucose (saccharification) from WHB expanded directly with incubation period till 60 h Among the various treatment, maximum yield of glucose and reducing sugars was recorded in 5.0 % moisture content followed by 7.5 % at 60 hours of incubation period. As the incubation time increased, the saccharification was altogether raised. Reducing

sugars at 5.0 and 7.5 % moisture content recorded 252.2 mg/g, and 262.0 mg/g individually. 123.1 and 132.0 mg/g of glucose was recorded in 5.0 and 7.5 % of moisture content treatment group.

Bioconversion of hydrolysate into ethanol

Complex polymeric substances in the tested biomass was effectively transformed into soluble sugars using soil isolates of co fungal strains *Trichoderma* sp and *Aspergillus* sp which could be used as substrate for ethanol fermentation. Respective fungal strain was distinguished by morphological characters like culture morphology, infinitesimal assessment of spores utilizing lactophenol cotton blue staining (Humber 1997). On PDA media, *Trichoderma* framed green conidia and scattered all in the plate (Fig. 4a). Microscopic assessment of conidia with lactophenol cotton blue uncovered 2.5 micron green globose shaped. *Aspergillus* sp framed thick dark elevated mycelia on PDA (Fig. 4b).Lactophenol cotton blue staining shows dull darker globose conidia. Conversion of complex polymers in the processed WHB into soluble substrate was carried out by solid state fermentation principles. Fungal based hydrolytic activity brought about soluble sugar substrate which was fermented into alcohol by ethanol fermenting strain. Maximum yield of total, reducing and non reducing sugars were recorded in processed WHB with 2.5 % of moisture content followed by 5.0 % (Fig. 5a).Soluble substrate thus obtained from WHB with different moisture content was fermented into ethanol under batch fermentation studies. Digested substrate got from WHB with 2.5 % moisture content uncovered maximum yield of ethanol (Fig. 5b,c).Total yield of ethanol was found to be 65.2 % with 77.3 g/l final concentration A continuous decrease in ethanol yield was seen in soluble substrate acquired at lower moisture content. Chinnathambi Pothiraj (2014) produced bioethanol from WHB that treated with cellulolytic fungal strains as in our present study.

Dye adsorption studies

Evaluation of dye adsorption efficacy of WHB was studied with methylene blue was carried out in this present investigation. To determine adsorption efficacy the experiment was done by varying the dosages of WHB followed by measuring the residual dye at different time periods (Fig. 5). UV visible spectra that depicts in Fig. 5c clearly indicates the characteristic changes of methylene blue spectra after WHB adsorption. It can be seen that all the tested dosages of WHB was found to be effectively adsorbed at early incubation time. Increase in dye removal (all the tested concentration) was noted in 30 minutes. Rate of adsorption efficacy was not affected significantly by the dosages of WHB (P = 0.05 %). Uniqueness of WHB was clearly inferred from its adsorption efficacy at its lower dosage. Percentage of COD removal was also found to be high in WHB treatment as shown in the Fig. 5d. Our present findings is in agreement with Kaur et al (2013) who reported the notable adsorption efficacy of WHB pre-treated with detergent (SDS).

Waste water treatment efficacy

Effect of WHB on the brewery waste water treatment was done based on evaluation of the various parameters such as alkalinity, total dissolved solids (TDS), chemical oxygen demand (COD) dissolved

oxygen (DO) biological oxygen demand (BOD), biofilm inhibition during 10 and 30 days after treatment. Batch studies were conducted to determine the waste water treatment efficacy (Fig. 6).All the tested parameters were found to be decreased in all the tested incubation periods and maximum reduction of all the parameters was recorded at 30 days of treatment (Fig. 6). Notable reduction of pH from 9.4 to 7.4 and 6.5,TDS 1860 to 782 to 431 mg/L, COD 1875 to 445 mg/L,BOD 980 to 311 mg/L TOC 453 to 132 mg/L,TN 32.4 mg/L to 9.5 mg/L,TP 36.4 to 10.0 mg/L was recorded in treatment (Fig. 7-13).The present findings clearly indicates that WHB treatment exhibited an effective water treatment efficacy by recording reduction of various physico chemical parameters as showed above. A gradual reduction of all the tested parameters was recorded at 30 days after treatment. Unique surface chemistry of polymers in the WHB and their interaction might be responsible for effective reduction of solid particles including soluble, insoluble ions. The evacuation of different heavy metals in the water utilizing WH has been examined broadly by Jonathan et al (2018). A pilot scale study led by them shows that promising evacuation of different substantial metals utilizing WH from a mild Northern Hemisphere stream contaminated water test and engineered arrangement. A report of Rita et al (2018) also reveals the potential waster water treatment efficacy of WHB. Followed by determination of physico chemical parameters, WHB induced biofilmicidal activity was studied. Biofilm formation in aquatic system also plays a major role in determining the water quality. Biofilm – microbial cells aggregates on the solid surface covered with polymeric matrix (Namasivayam et al 2019). Crystal violet spectrophotometric assay was carried out to determine the anti biofilm effect of WHB which was studied by measuring the optical density of extracted adherent biofilm. Results shows that biofilm inhibition percentage was found to be high in WHB treatment (Fig. 14). Morphological changes in the biofilm architecture was studied by SEM analysis which demonstrates the marked effect of WHB on the biofilm. SEM micrograph that depicted in the Fig. 15 shows the noteworthy impact of WHB on the biofilm architecture. Weakening or structural damage of the biofilm after the exposure to the natural phytochemicals components that may present in the WHB was clearly inferred the SEM analysis.

Reusability of WHB treated water and dye

Reusability of WHB treated effluent, methylene blue adsorbed water was studied by determination of phytotoxicity. Determination of seedling emergence or germination index in percentage is a simple method to study the phytotoxic effect (Tiquia and Tam, 1998).In this study, WHB treated effluent, dye adsorbed water exhibited no significant effect on the seedlings emergence. Samples derived from WHB treatment and methylene blue adsorbed water recorded 100.0 % seedlings emergence. Untreated –control group of waste water and methylene blue reveals 87.0 and 76.0 % of germination index respectively. (Fig. 16).

Evaluation of non target toxicity studies on zebrafish model

Biocompatibility study was carried out by determination of developmental toxicity studies on zebra fish. Among the various models, Zebra fish (*Danio rerio*) is most commonly used to check the biocompatibility due to its diverse salient features. The rapid development of zebrafish compared with other animal

models make it a great advantage for assessing developmental toxicity (Bailey et al 2013). The transparency during embryo stages makes it easy to observe toxic effects, such as lethality, reproductive toxicity and teratogenicity (Casreo et al 2018). In addition to developmental similarities and genetic homology, there are morphological and physiological similarities in major organs, aside from the lung and mammary glands, which are not present in zebrafish .In this study, the biocompatibility of WHB treated methylene blue and brewery water was checked by measuring the notable changes in the embryonic stages at different time periods (Fig. 17). No mortality and structural changes or abnormalities were recorded in WHB adsorbed water and methylene blue treatment which clearly reveals the biocompatibility.

Growth stimulation efficacy

Pot experiments was carried out to determine the plant growth stimulation efficacy of WHB against *V.mungo*. Plant growth promotion effect was considered by estimating the length of the shoot, leaf surface territory, stature of the plant, all out new branches rose/plant ,all out foliage tally/plant, complete chlorophyll includes of the leaves in the particular treatment groups. Our present examination is in concurrence with Lata and Veenapani (2013), Vidya and Girish (2014) who revealed the plant growth promotion efficacy of WH. Mix of WHB with the bacterial biofertilizers additionally concentrated to evaluate the similarity. Results demonstrates that WHB alone and its blend with biofertilizers showed vital contrasts on all the tried plant development parameters (Fig. 18, 19).In both tried timeframes, WHB inoculated plants had high shoot length, increasingly number of branches, foliage density with high chlorophyll content. WHB likewise displayed the best similarity with all the tried biofertilizers by showing the improved\ execution of immunized plants. Results showed that all the tried plant development parameters were expanded considerably when WHB joined with the particular tried biofertilizers than when WHB and separate biofertilizer utilized alone. WHB-R+P+A treatment uncovered vital impact on all the tried parameters which demonstrated to positively affect execution of tried plant accordingly in high pace of supplement accessibility.

Growth pattern of *B.bassiana* cultured in WHB liquid medium and its influence on survival parameters of *A.janata*

Pesticidal activity of biopesticides is mainly dependent on satisfactory production and viability of inocula. A few endeavors have been made all through the world to mass produce biocontrol agents by utilizing diverse media (Sahayaraj & Namasivayam 2007). Among the diverse fungal biopesticides, *Beauveria bassiana* – hyphomycetes strain is an important bioccontrol agent has been utilized as an insecticidal agents against economic important pests (Tuncilley et al.2004). B. bassiana used in the present examination was isolated from soil sample by the use of highly selective medium as described in our recent studies. Dry ,fine particular white shaded aerial and substrate mycelial fungal colonies as shown in the Fig. 20a. dry, single celled, oval conidia (Fig. 20b) by lactophenol cotton blue staining reveals the specific confirmative parameters of *B.bassiana*. Isolated fungal strain was assessed for biomass production including spore yield. Culture medium prepared with various conentration of WHB

was assessed for mass augmentation of *B.bassiana* and the impact of media on biomass yield, spore creation and pesticidal movement against *Achaea janata*. WHB based medium was set up by suspending distinctive convergence of very much homogenized WHB (0.1%,0.25%,0.5%,1%,2%,2.5%,5%) in 100 ml of refined water (Fig. 21). Results demonstrates that diverse centralization of WHB enhanced media upheld development and sporulation of *B.bassiana* (Fig. 22).A progressive increment in biomass yield was seen in expanding focus .nonetheless, most extreme yield of biomass was recorded in 0.75 % fixation followed by 1.0 %. Supply of fundamental and basic supplement factors from the prepared biomass remove in the medium set off the remarkable development and sporulation of the tried fungal strain. Normal spore size of tested fungi in the individual treatment bunch uncovered 1.91-2.73 micron (Fig. 23). Media with various conentration of WHB upheld spore germination and the pace of germination was seen as most extreme in 0.75 % and 1.0 % (Fig. 24). Microscopic assessment demonstrates the germinated spores of *B.bassiana* derived from WHB medium (Fig. 25). Medium with all the grouping of WHB recorded sprouted spores. in any case, pace of germination of spores into hyphae with remarkable number was seen in 0.75 and 1.0 % WHB. Usage of WHB as an elective substrate for biocontrol specialists not yet announced. As per our best information, WHB based fluid media utilized in the present investigation as an elective substrate for mass augmentation of fungal biopesticidal specialist is our first report. Since WH is accessible liberated from cost, this innovation is monetarily feasible and furthermore helps in the annihilation of this problematic oceanic weed.

Pesticidal activity of the spores of *B. bassiana* got from WHB media was contemplated against larval instars of castor semi-looper Achaea janata In various phases of *A.jenata*, the larval mortality was seen ahead of schedule after the utilization of spores and the days therefore expanded when exposed to resulting spore fixation and instars. Insecticidal efficacy was dictated by recoding aggregate mortality of particular larval instars presented to various of contagious spores at various time interims. From the total mortality data, lethal conentration 50 (LC 50) was surveyed. Probit examination was utilized to decide LC 50 and the qualities were appeared in Table 1. Among the different gauge of the relapse based probit examinations, the chi-square trial of the bioassay indicated homogeneity of the test populace which is an impression of a solid match of the watched and anticipated reaction. Results shows that the progression of larval stages decided the LC50 esteem.

Table 1
Effect of *B. bassiana* spores on LC_{50} of *A.janata* larval instars

Instars	Regression equation Y = a + bx	LC_{50} Spore / ml	Variance	Chi-square value	Fiducial Limit (95% confidence)	
					Lower limit	Upper Limit
I	2.182 x – 0.32	2.01 X 10^2	0.121	1.021	1.12X 10^2	3.11x10^3
II	2.592 x – 3.22	12.02 X 10^2	0.201	1.143	10.1X10^2	17.2X10^2
III	1.693 x – 0.42	31,4 X 10^3	0.423	2.321	15.2X10^3	43.2X10^3
IV	1.401 x + 0.11	43.3X10^4	0.512	4.123	21.2X10^4	5.0X10^5

Results shows that all the larval instars of *Achaea janata* were susceptible to the spores.(Fig. 26-30). As the instar propelled, a lessening in mortality and an expansion in time for beginning mortality were recorded. Among the four instars, complete mortality was seen in first and second instars at most extreme measurements. On account of third and fourth instars there was a decrease in the level of mortality as for time and measurements of the spores utilized. Onafre et al (2002) reveals that fungal spores treatment completely shut down the metabolic activity of the insect which in turn causing death. The present investigation would recommend the conceivable usage of WHB as a media for the mass increase of biocontrol specialists with high potential biocontrol action.

Conclusion

The use of aquatic weeds biomass for the production of various value added products is now being extensively studied in the various parts of the world. In this study, eco friendly bioconversion method was adopted for the utilization of alkali pre treated WHB as the substrate for biotechnologically important products. WHB that treated with alkali brought about bioethanol with notable yield, strength. Marked effect on the plant growth promotion effect, waste water treatment efficacy coupled methylene blue dye adsorption reveals the effective bioresource utilization and control or management strategy of aquatic weeds via green route. From these findings, we would propose the guidelines of WHB can be recommended as costless, profitable and eco altruistic framework for potential biotechnological application.

Declarations

Acknowledgement

We acknowledge National Agro foundation for the chemical analysis.

Conflicts of interest

We declare that no conflicts of interest

Funding

This research did not receive any specific grant from funding agencies in the public ,commercial, or not-for profit- sector

Conflicts of interest

We declare that no conflicts of interest

Availability of data and material (data transparency)

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Code availability (software application or custom code)

Not applicable to this study

Authors' contributions

Equally contributed

Ethics approval (include appropriate approvals or waivers)

Not applicable to this study

Consent to participate (include appropriate statements)

Not applicable to this study

Consent for publication (include appropriate statements)

Not applicable to this study

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Figures

Figure 1

Collection and processing of water hyacinth (a,b- Sampling sites, c,d,e- collected, processed WHB)

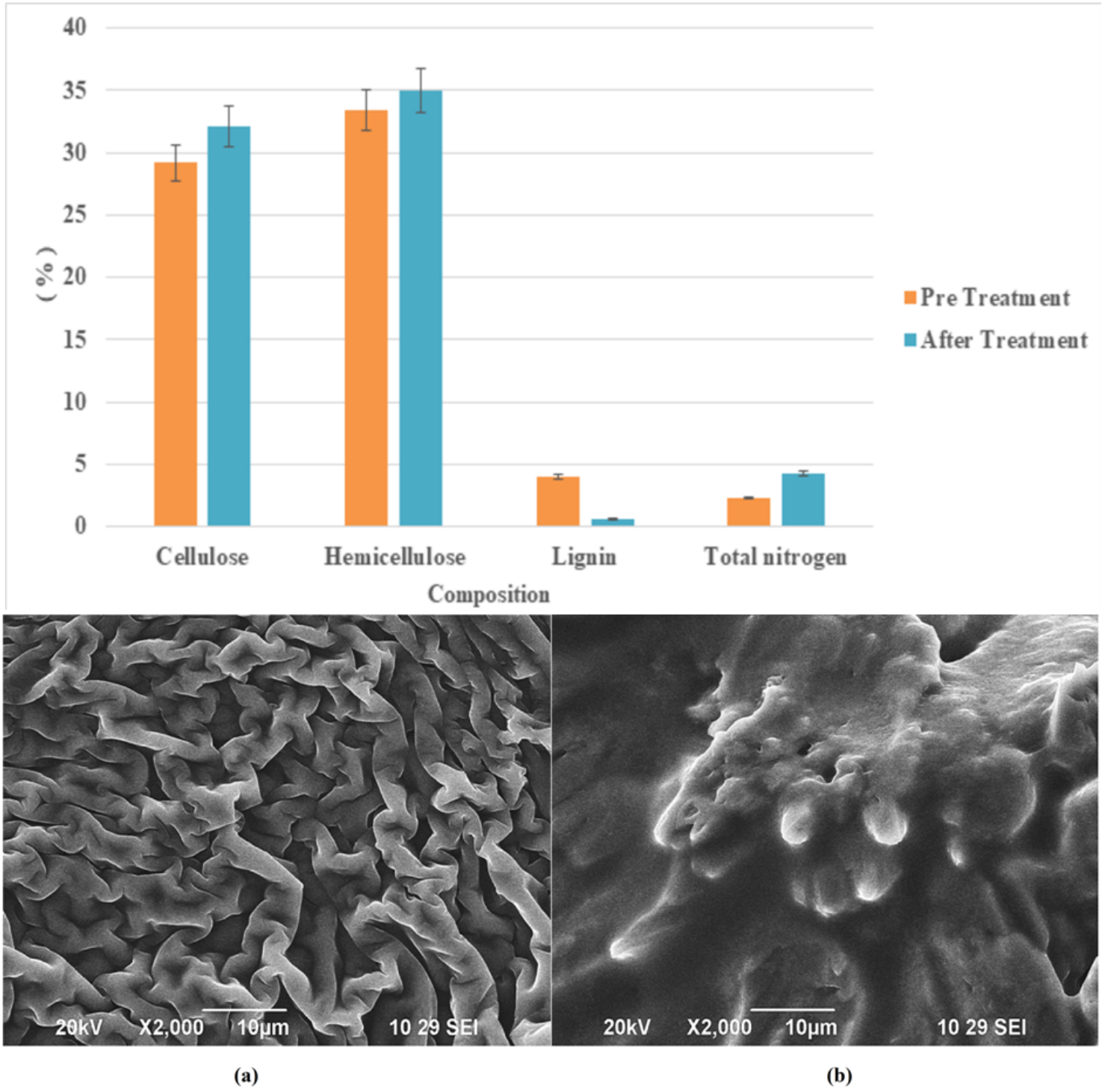


Figure 2

2a.Effect of alkali treatment on the chemical composition of water hyacinth

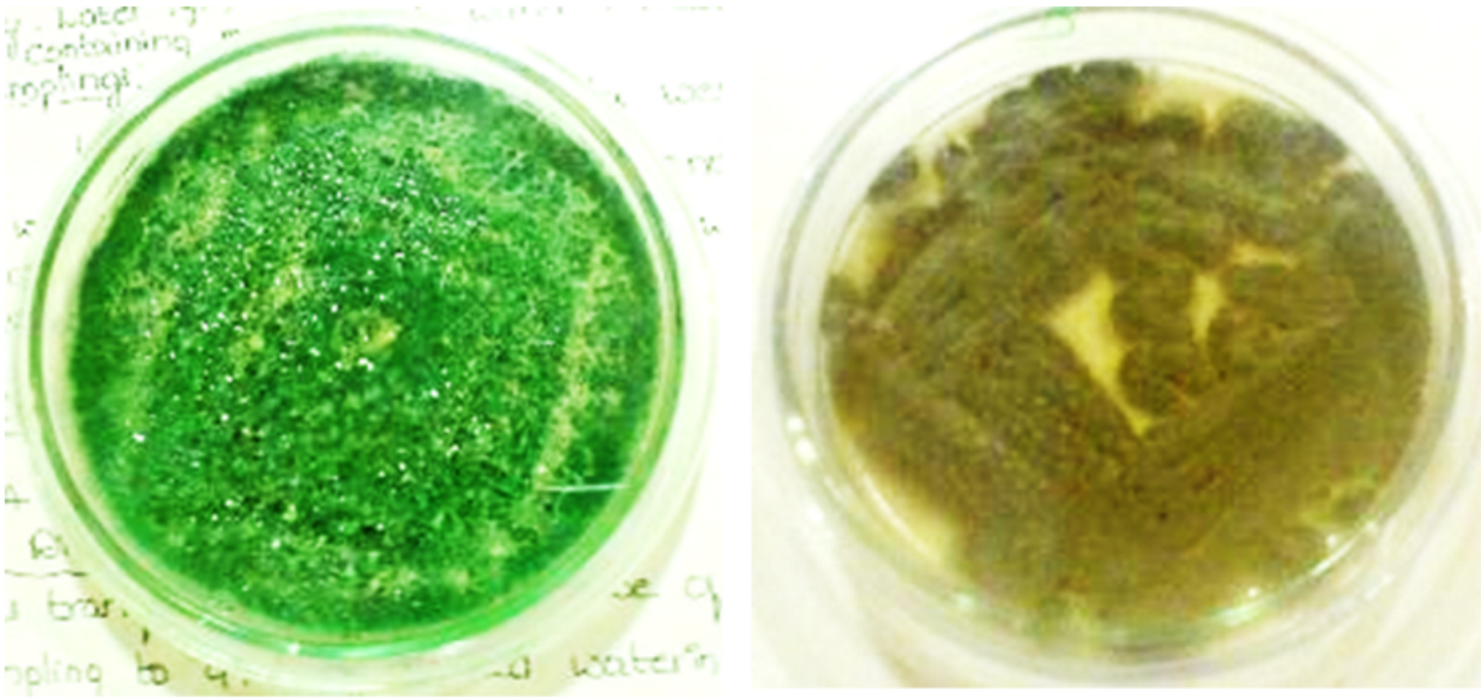


Figure 3

Cellulolytic fungal strains on PDA plate (a) Trichodermasp (b) Aspergillus niger

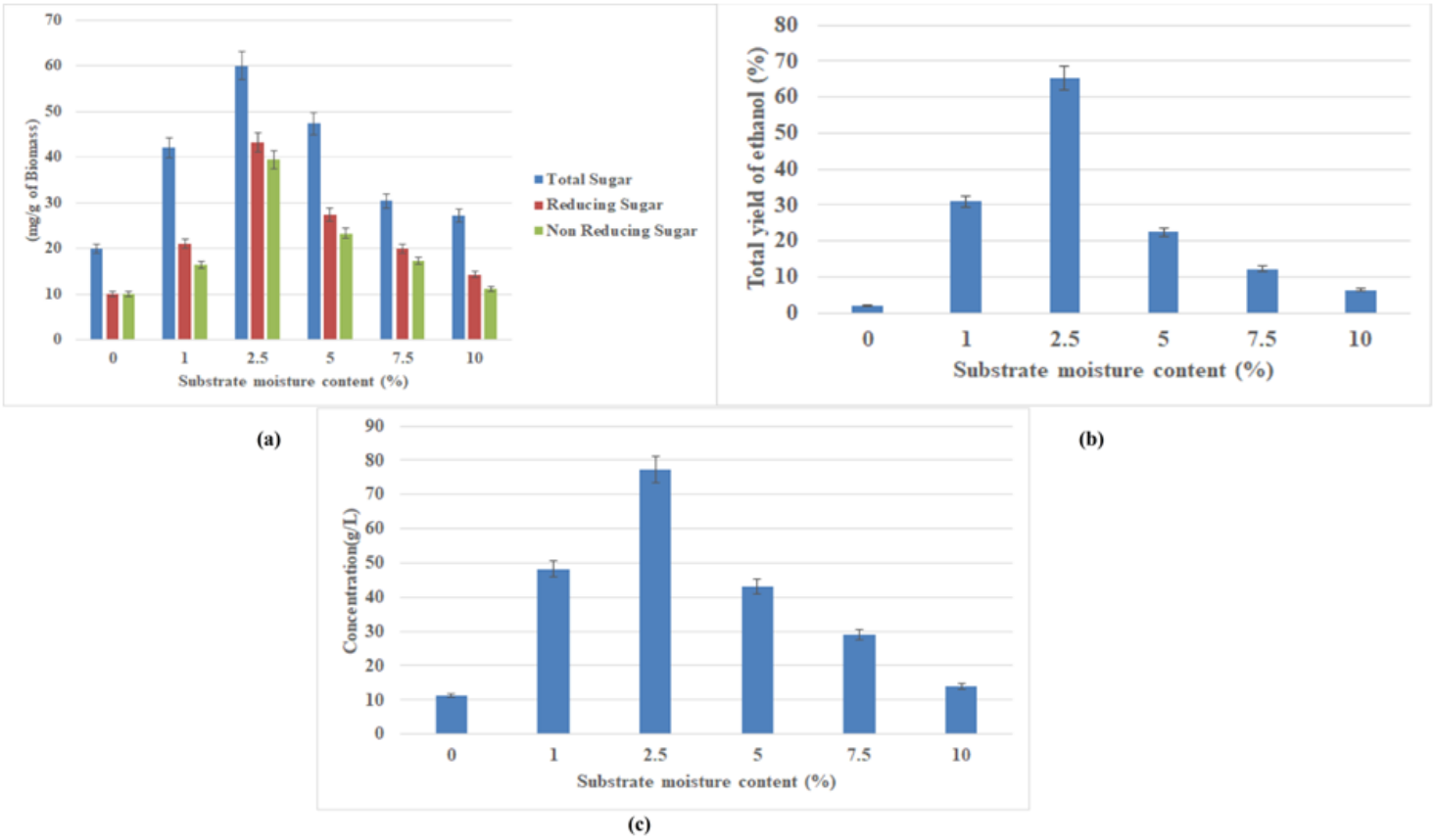


Figure 4

Saccharification and bioethanol production (a).Effect of moisture content on total, reducing and non reducing sugar of enzyme hydrolyzed WHB (b).total yield of ethanol (c).Concentration of ethanol

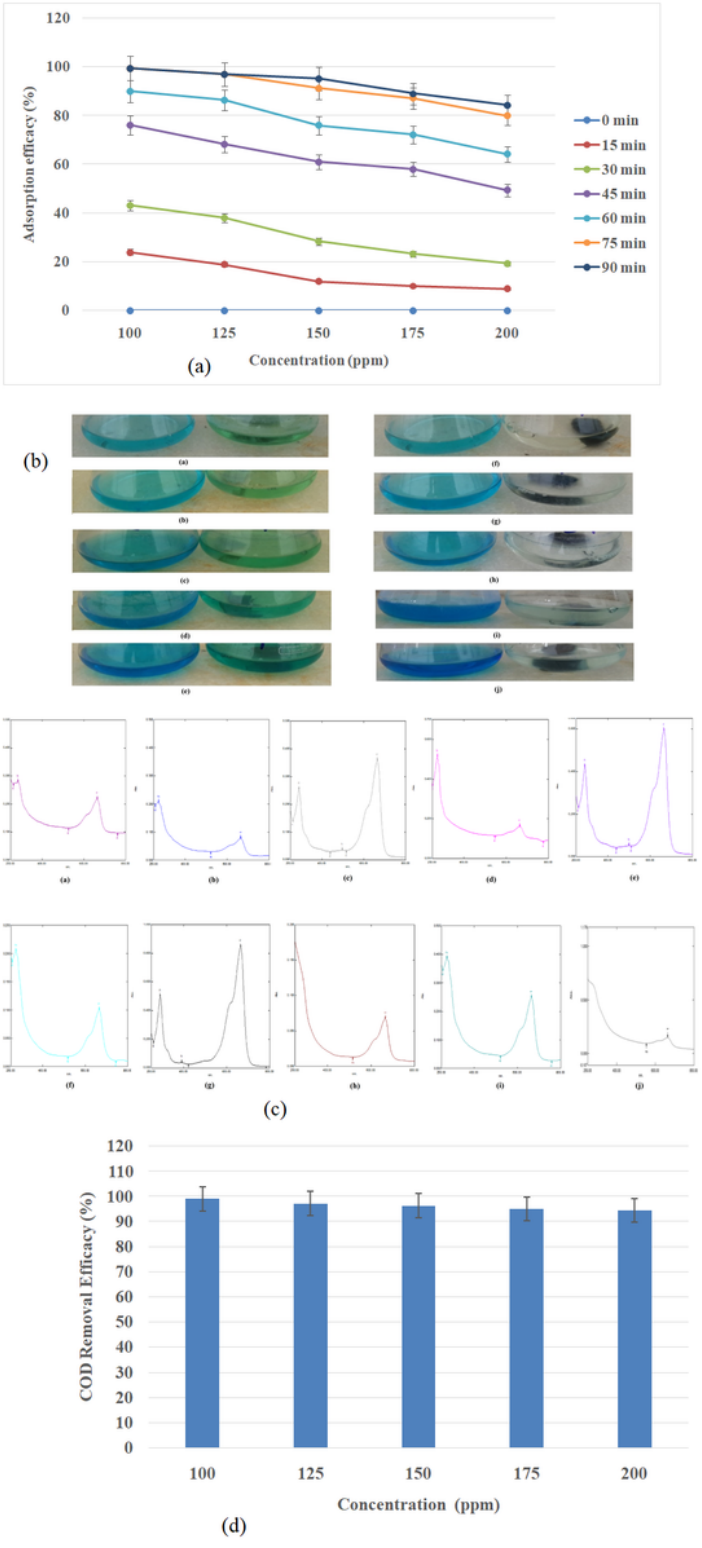


Figure 5

Efefct of WHB on the adsorption efficacy of methylene blue (a) b. Reaction mixture reveals the adsorption efficacy of methylene blue by WHB at different time intervals c. Changes in UV visible absorption spectra of methylene blue adsorbed by WHB d. COD removal efficacy of methylene blue

(a)

(b)

Figure 6

Waste water treatment efficacy ofWHB (a) 15 days *b) 60 days

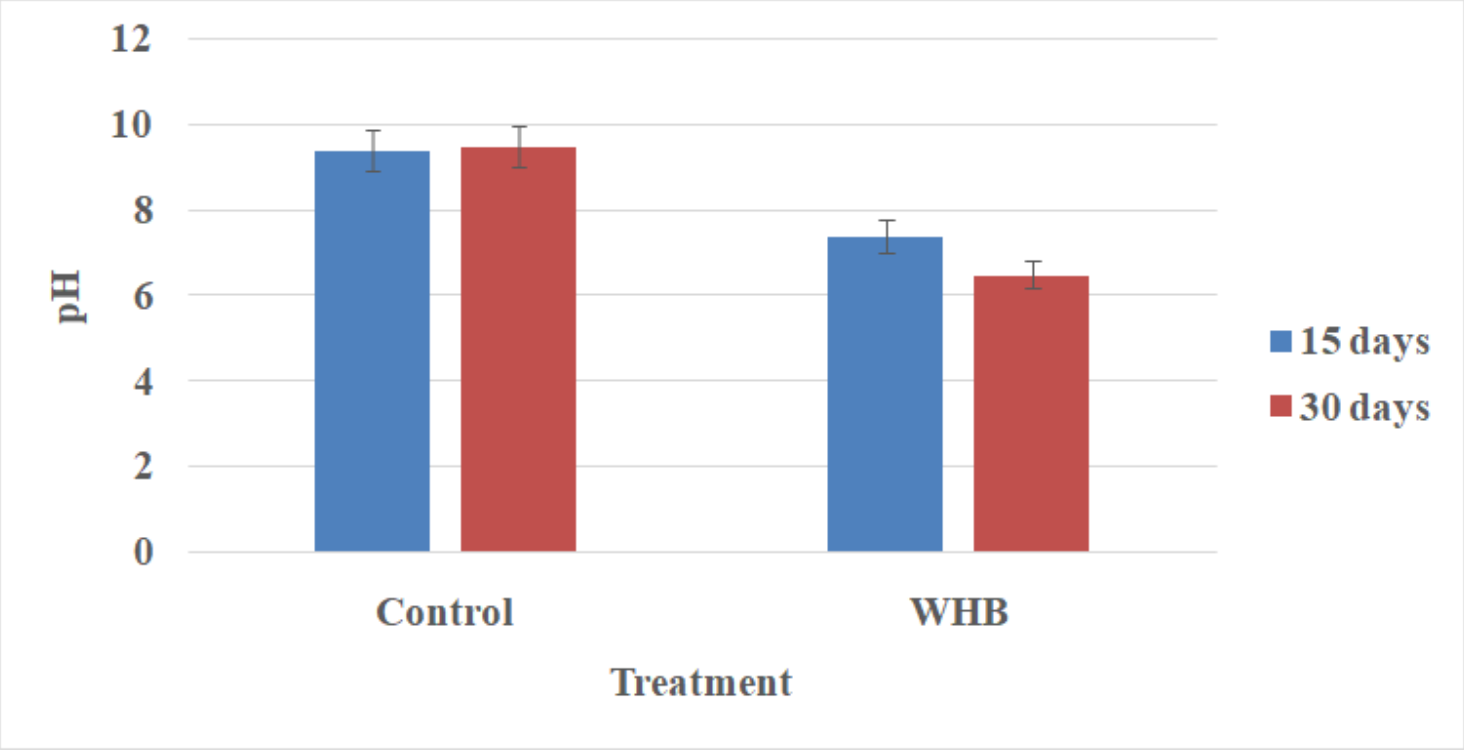


Figure 7

Effect of WHB on pH of brewery waste water

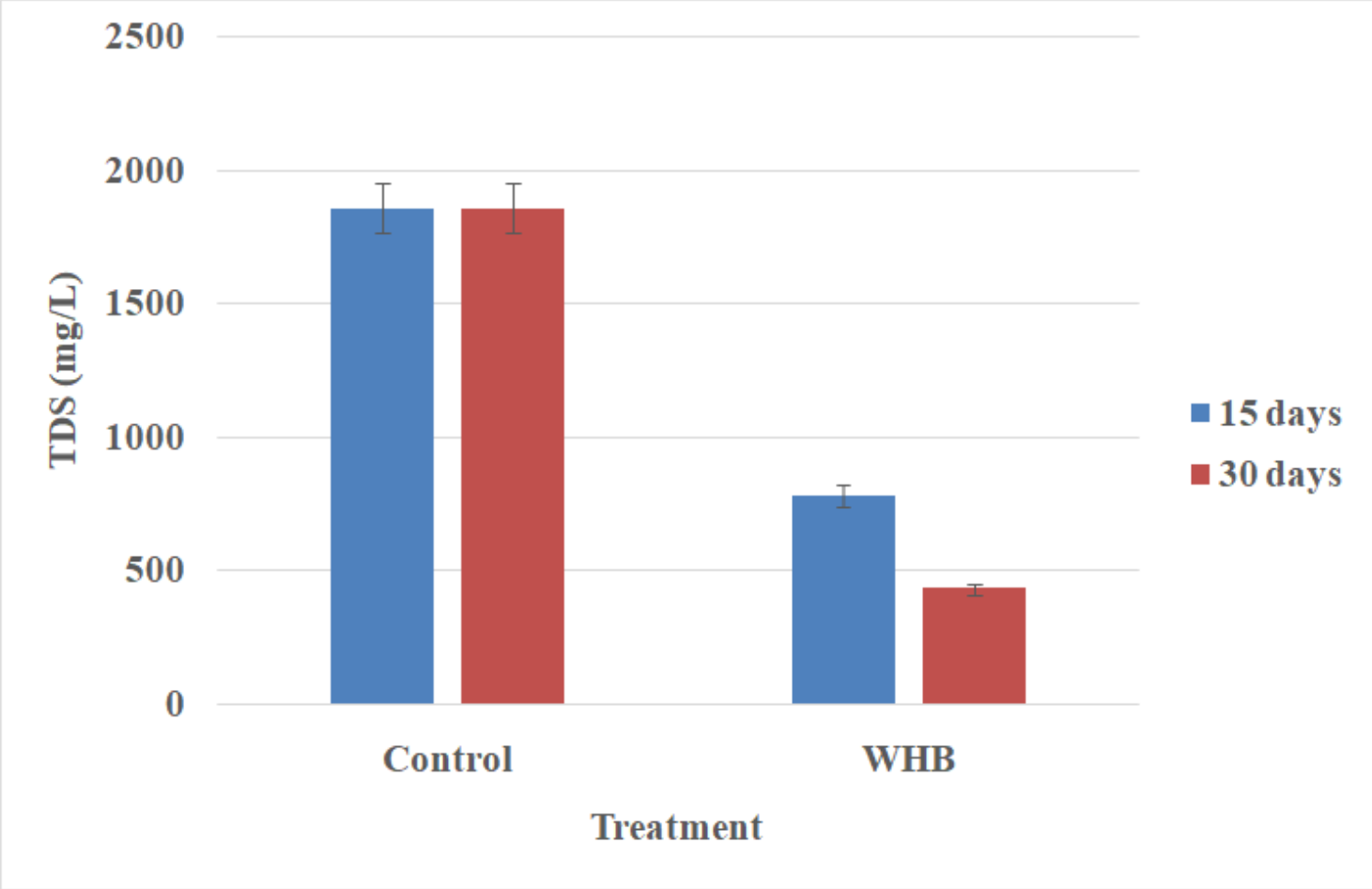


Figure 8

Effect of WHB on total dissolved solids (TDS) of brewery waste water

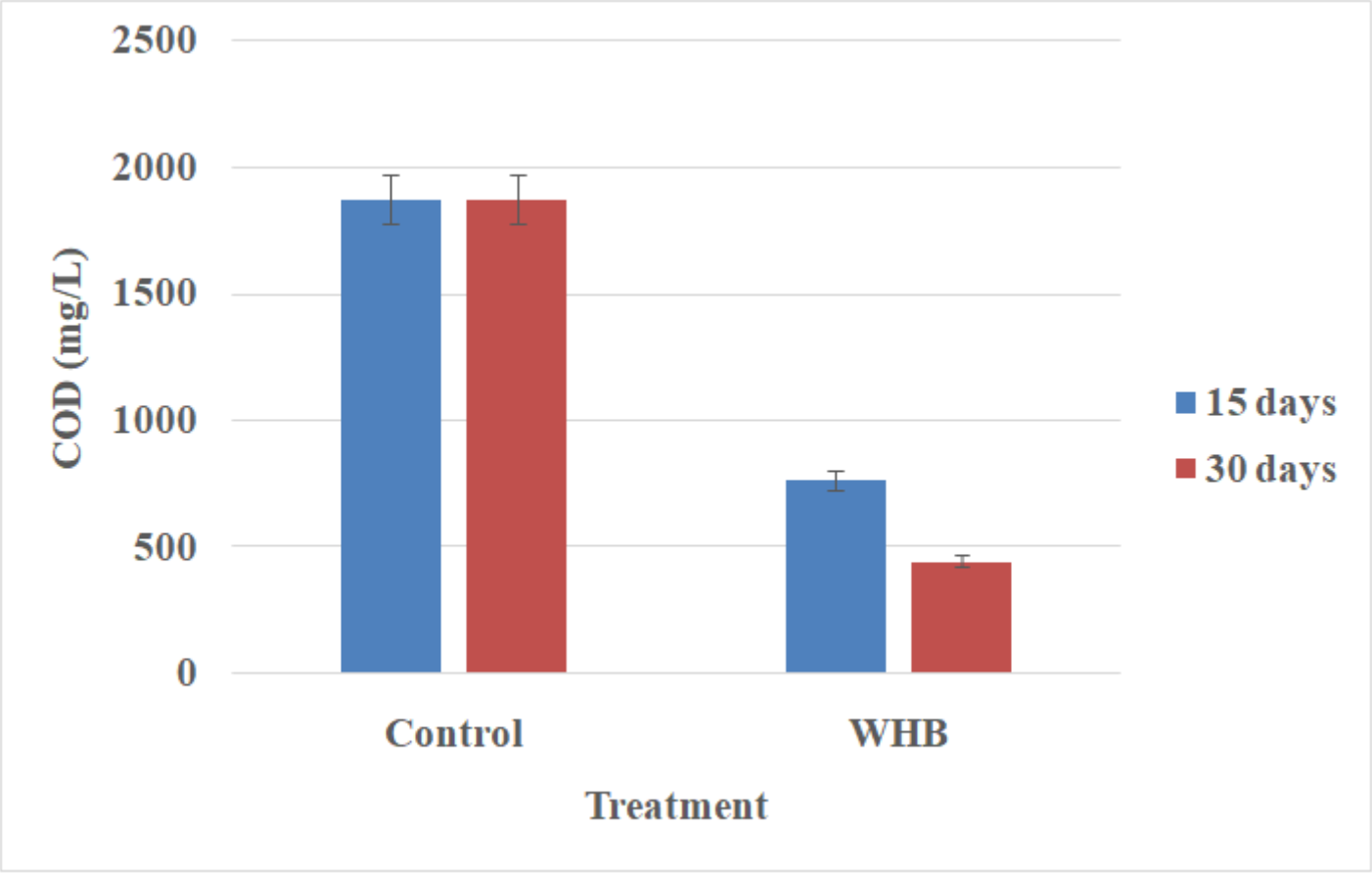


Figure 9

Effect of WHB on chemical oxygen demand (COD) of brewery waste water

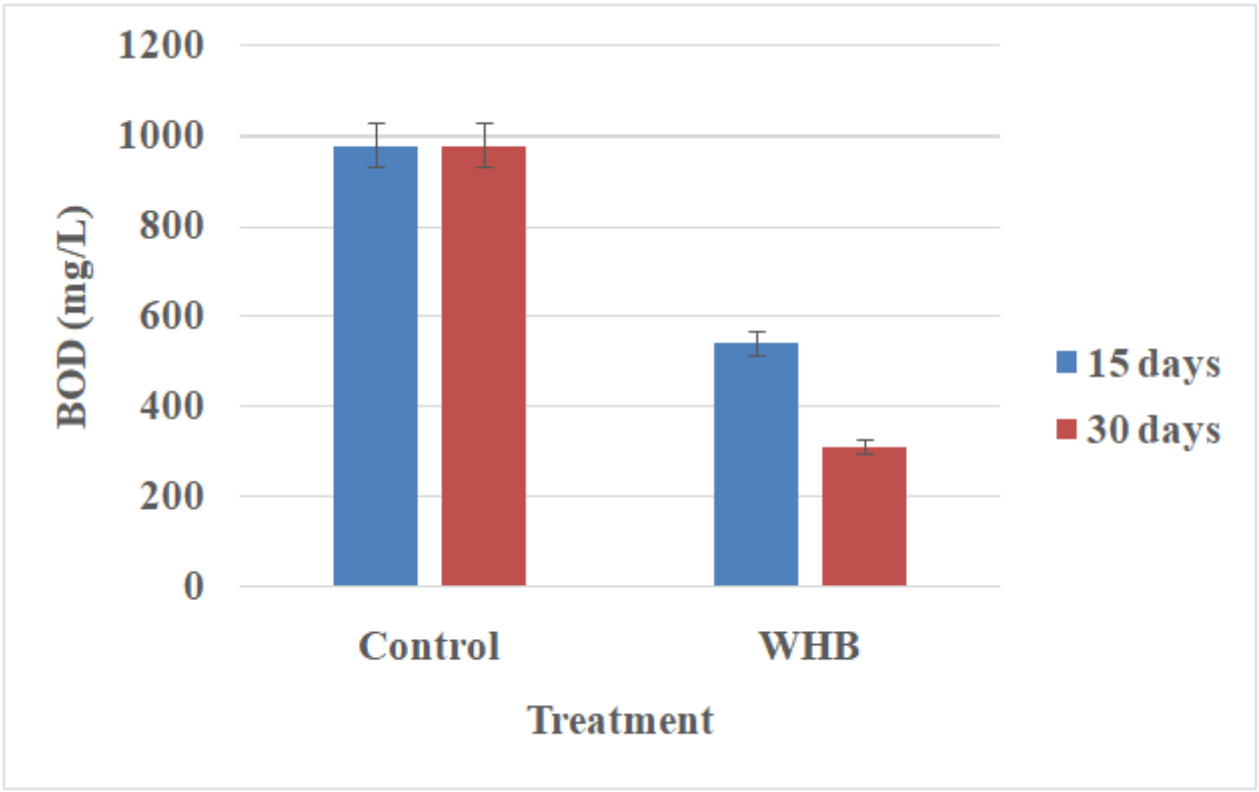


Figure 10

Effect of WHB on biological oxygen demand (BOD) of brewery waste water

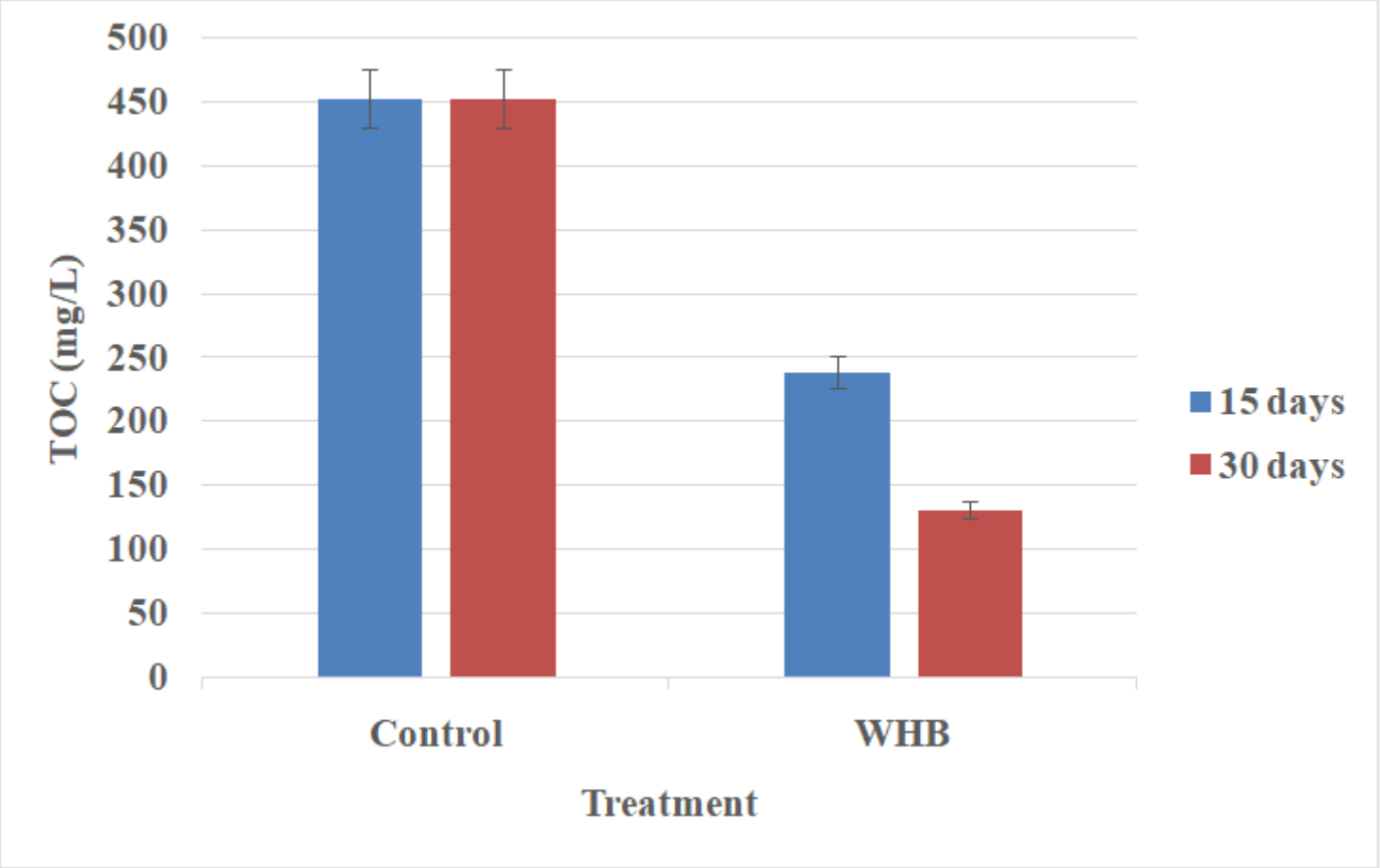


Figure 11

Effect of WHB on total organic carbon (TOC) of brewery waste water

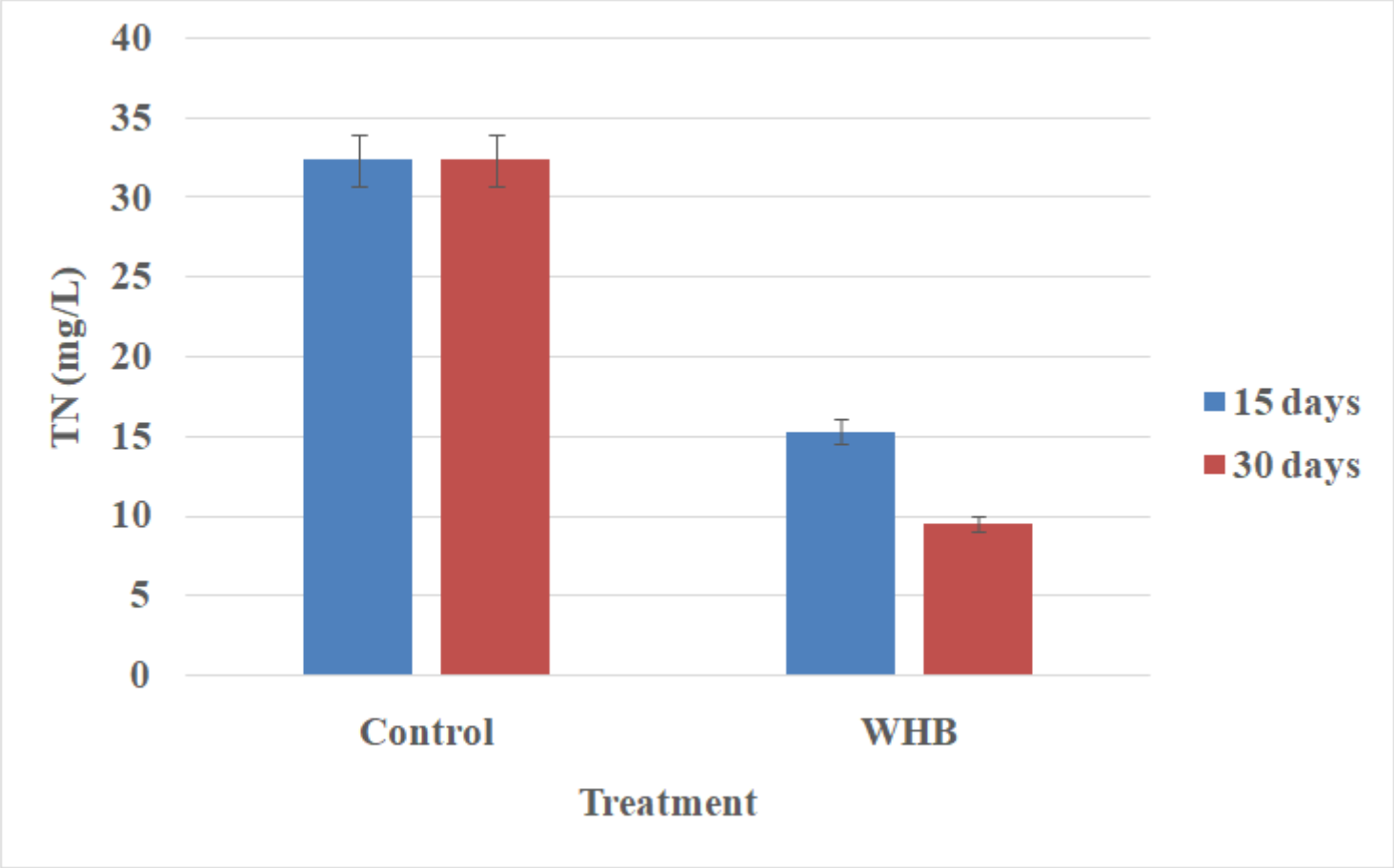


Figure 12

Effect of WHB on total nitrogen (TN) of brewery waste water

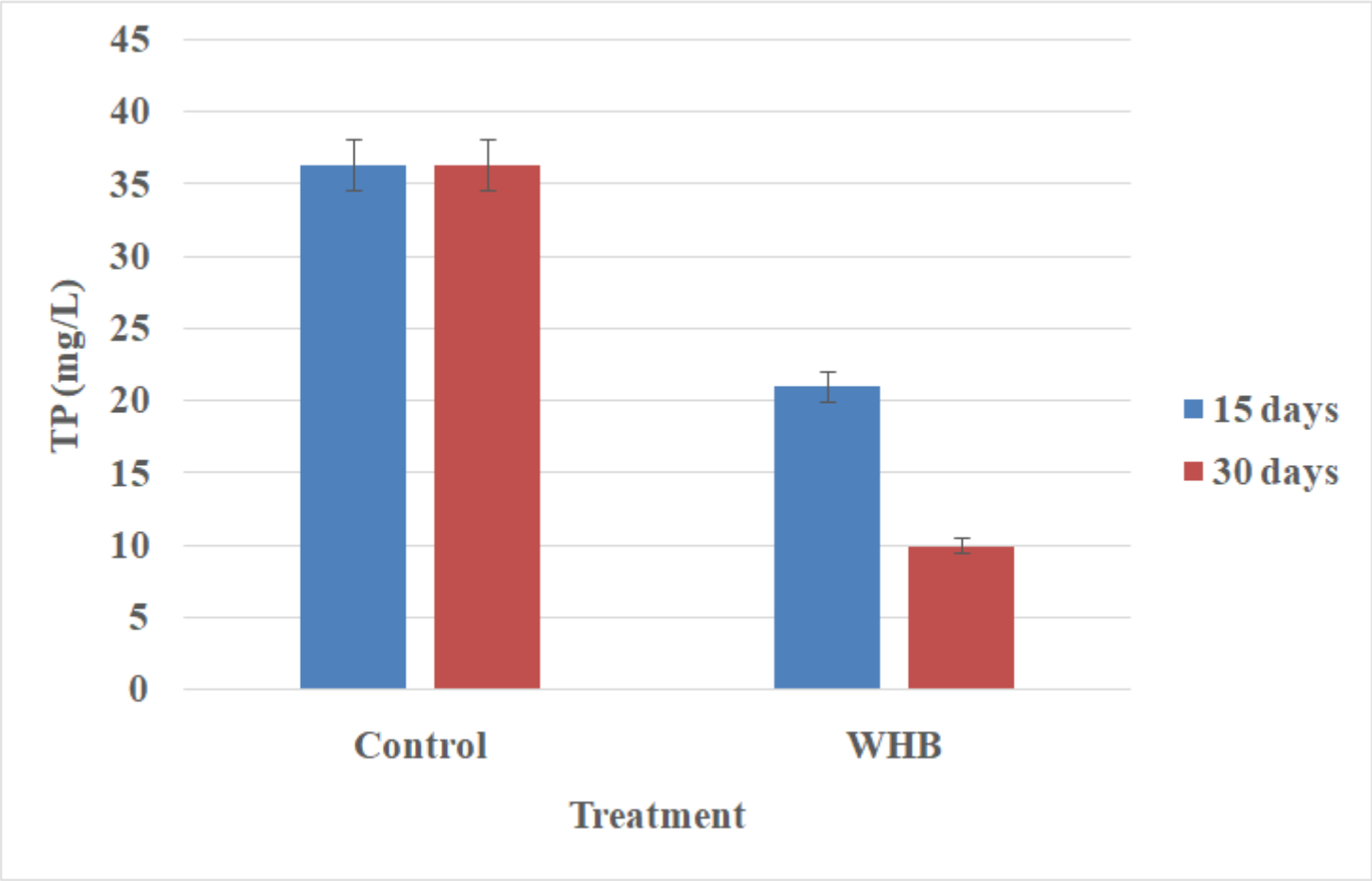


Figure 13

Effect of WHB on total phosphate (TN) of brewery waste water

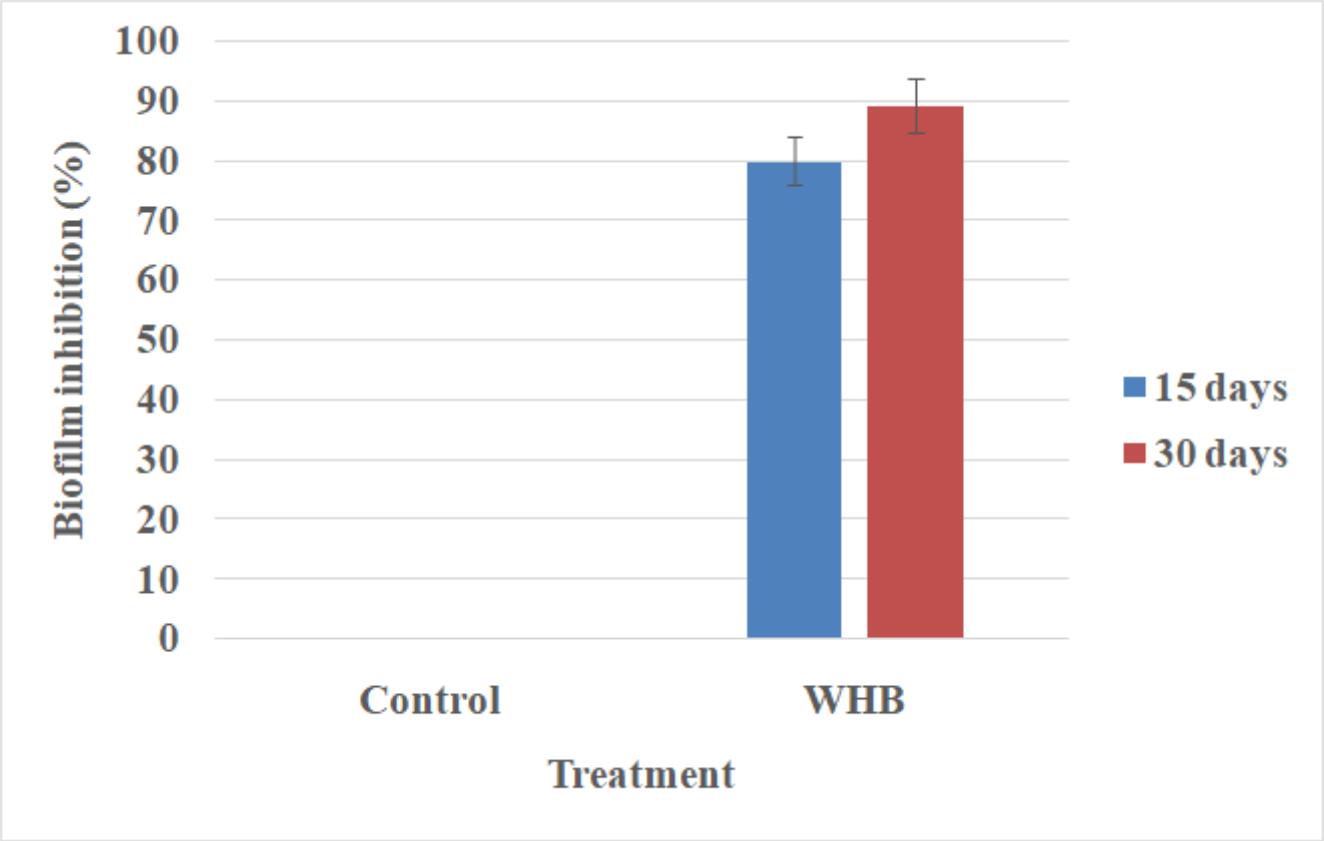


Figure 14

Biofilm inhibition of WHB

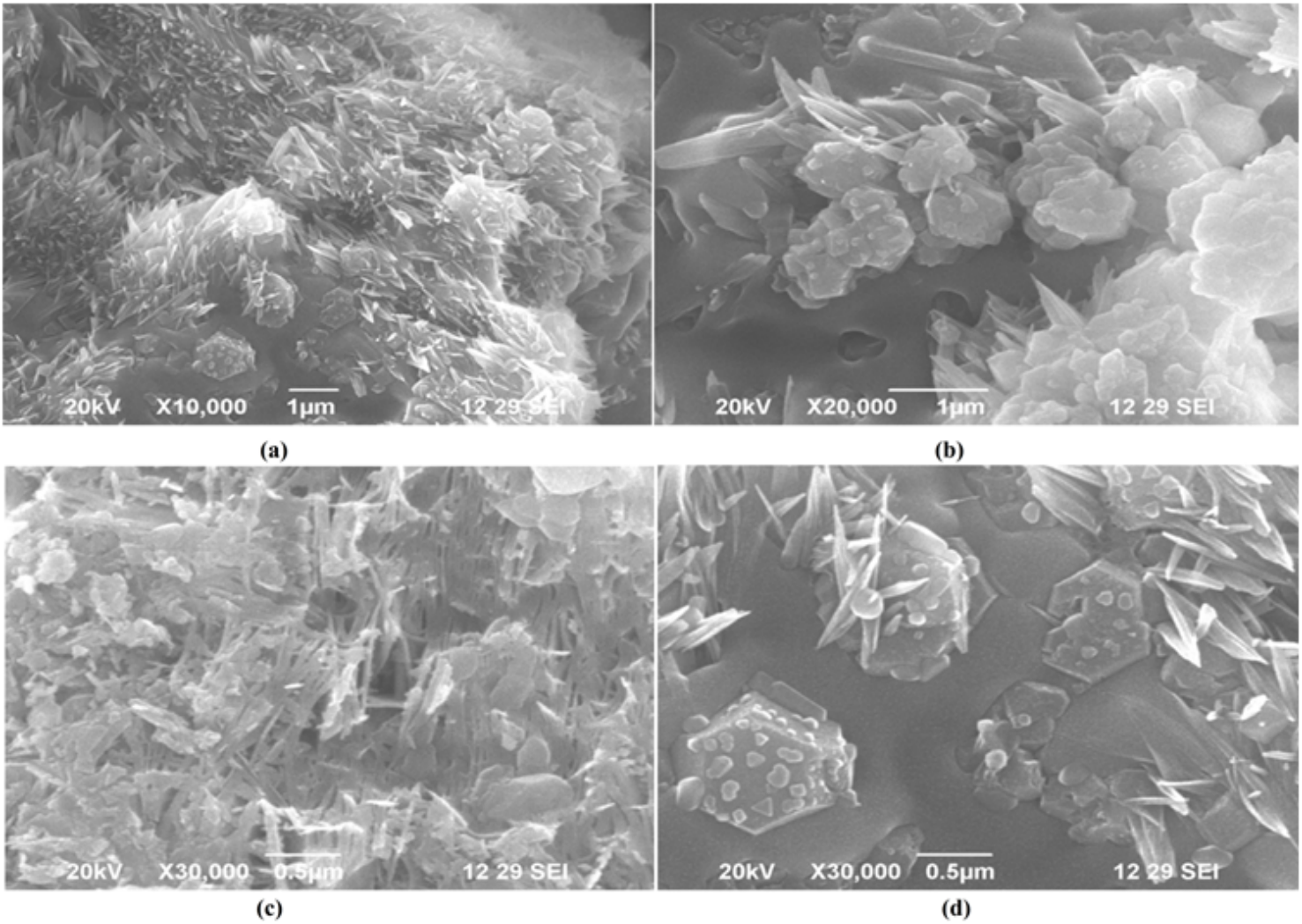


Figure 15

SEM micrograph of biofilm treated with WHB

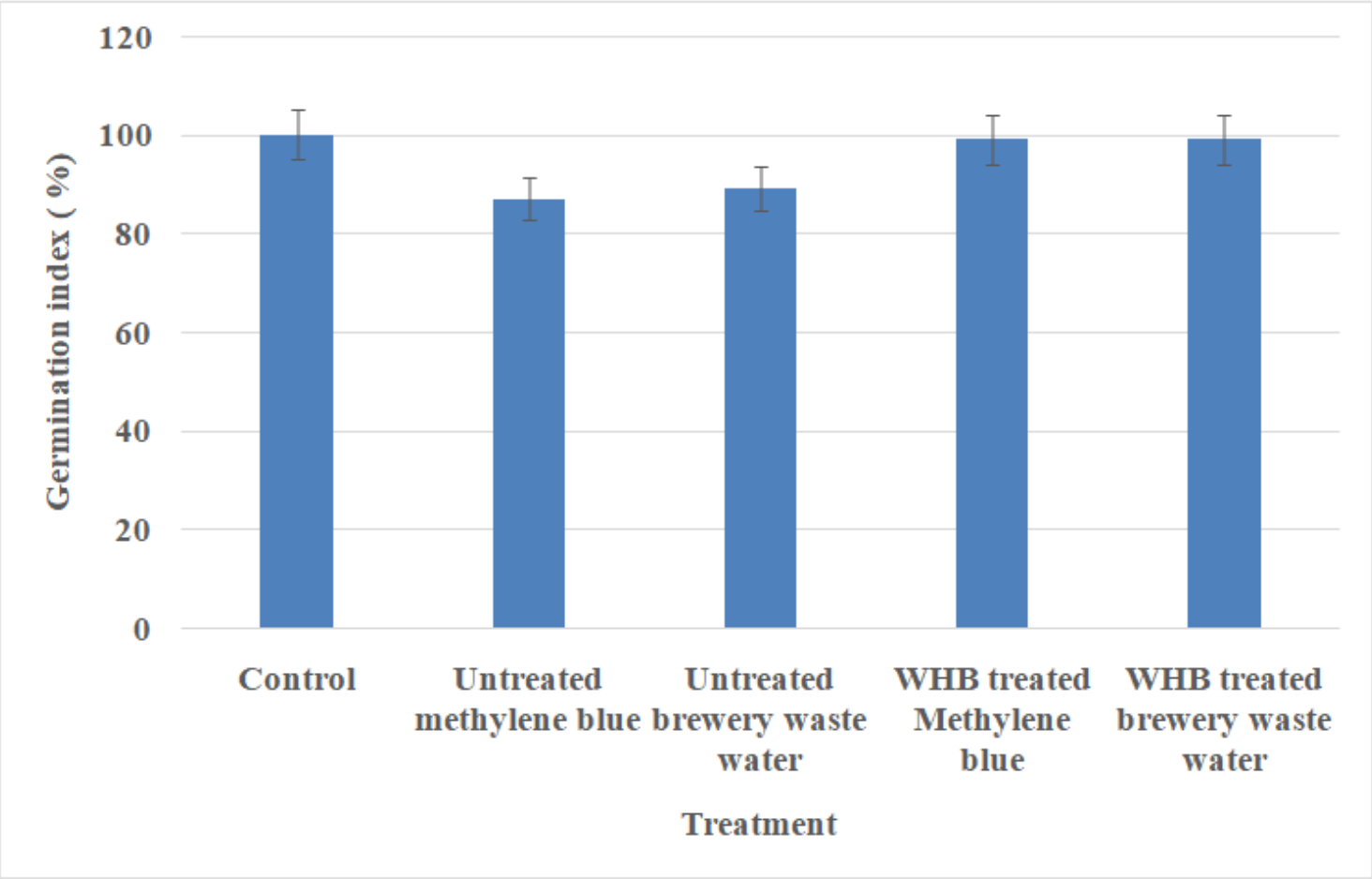


Figure 16

Effect of WHB treated dye & waste water on the germination index of V.mungo

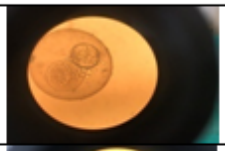
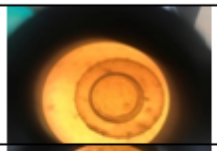
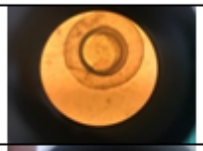
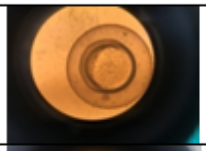
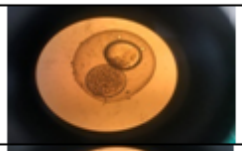
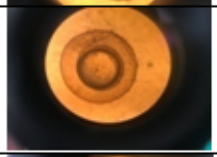
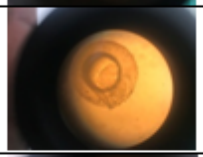
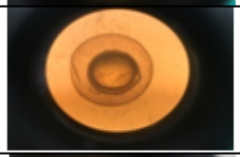
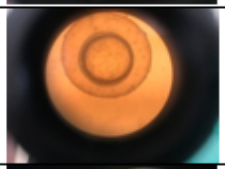
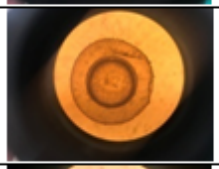
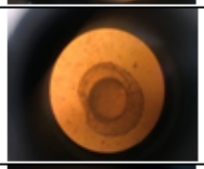
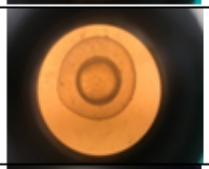
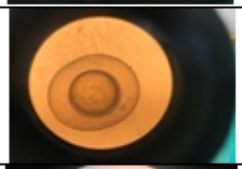
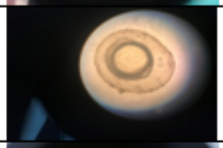
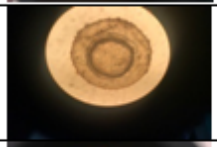
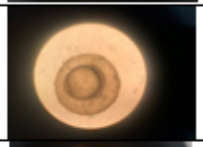
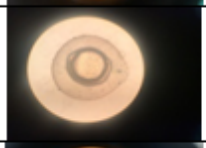
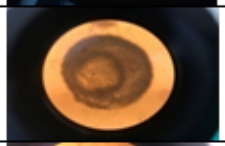
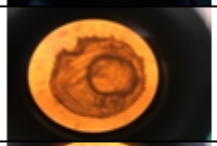
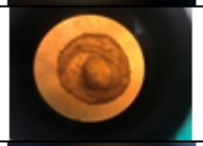
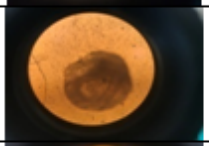
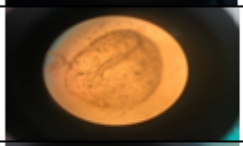
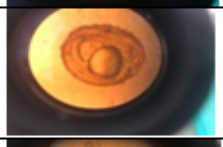
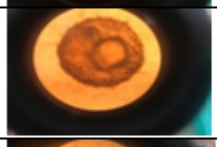
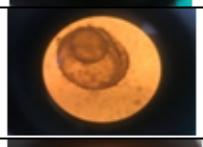
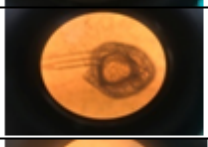
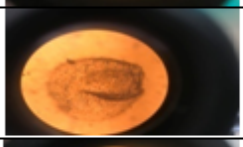
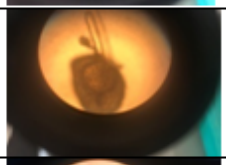
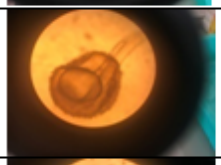
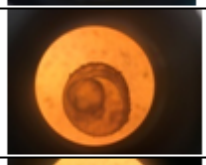
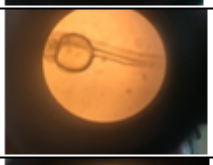
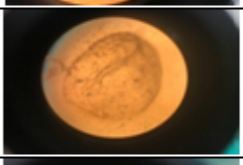
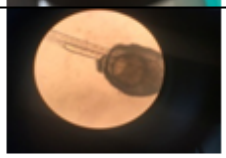
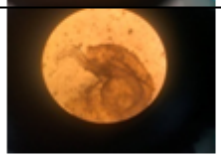
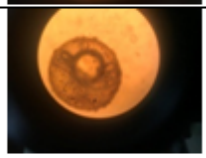
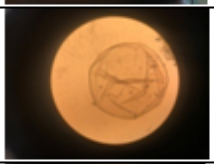
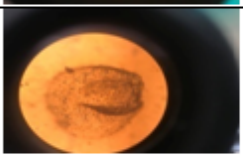
S. No	Time (hours)	Control	WHB treated methylene blue	WHB treated brewery waste water	Untreated waste water	Untreated methylene blue
1	12					
	16					
	20					
	24					
2	30					
	36					
	42					
	48					
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Figure 17

Microscopic examination of treatment groups of zebrafish embryo of different developmental stages

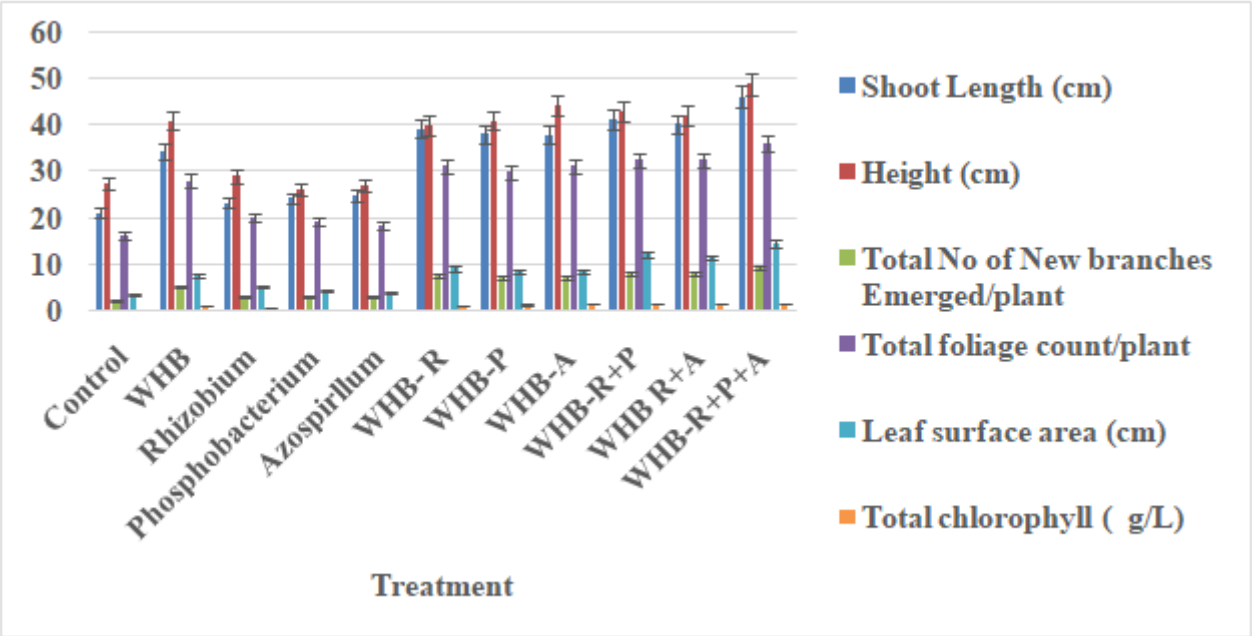

Figure 18

Plant growth promoting effect of WHB alone and bacterial biofertlizers on V.mongo – 30 days after treatment

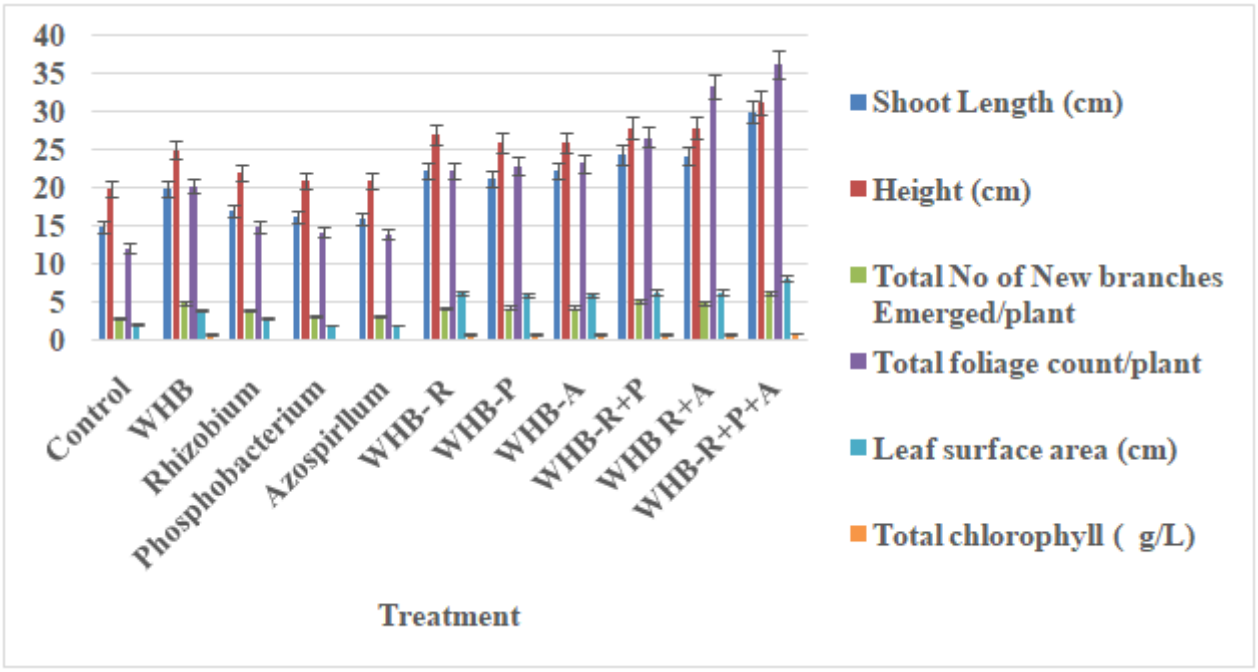

Figure 19

Plant growth promoting effect of WHB alone and bacterial biofertlizers on V.mongo – 60 days after treatment

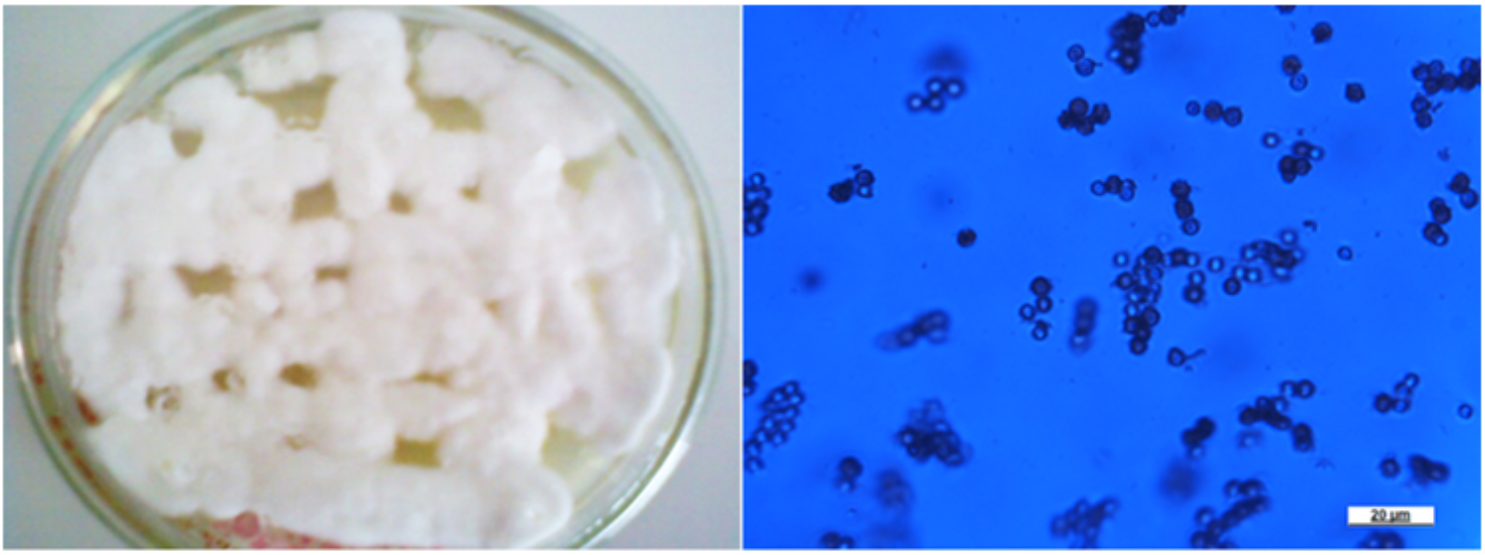

Figure 20

Beauveriabassiana culture on PDA plate (a)Microscopic examination of spores stained with lactophenol cotton blue

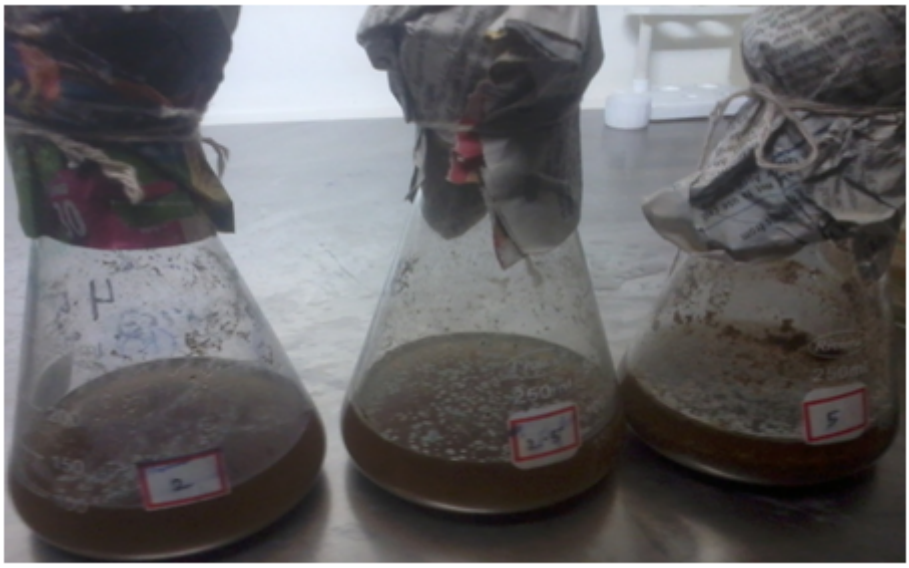

Figure 21

In vitro mass multiplication of B.bassiana in WHB media

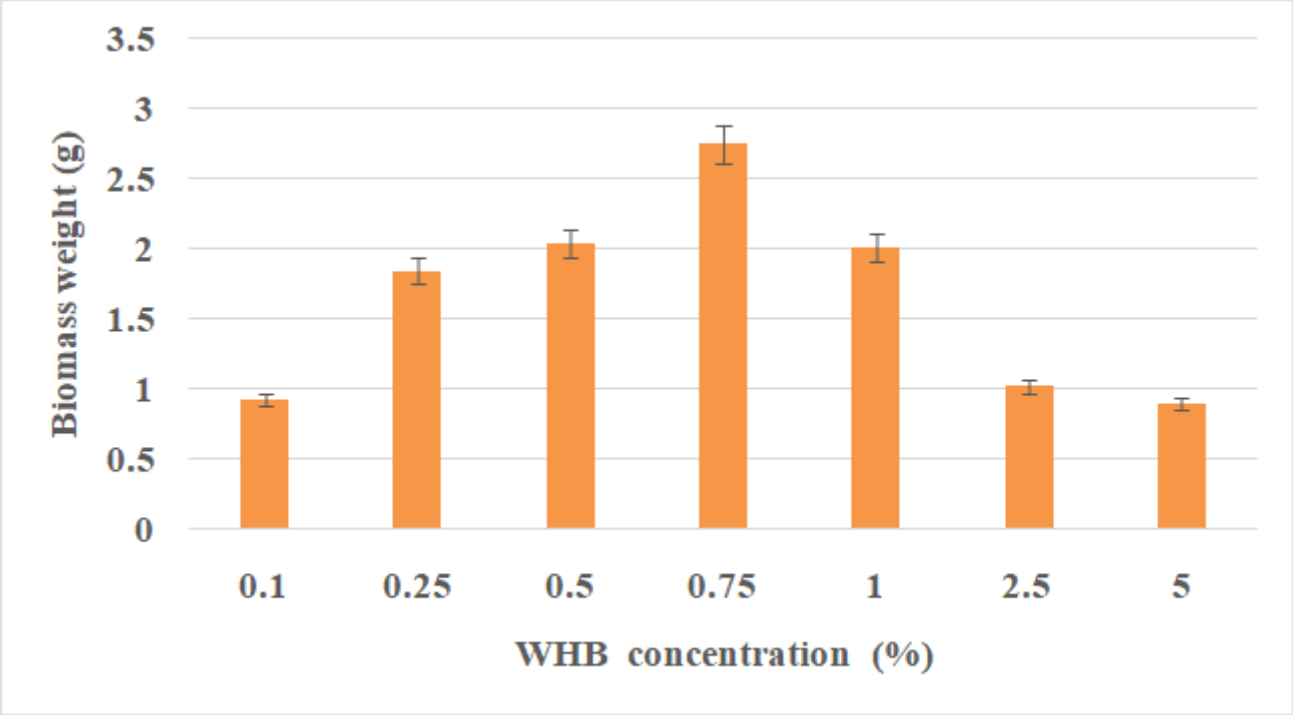

Figure 22

Effect of WHB media (%) on the biomass of B.bassiana

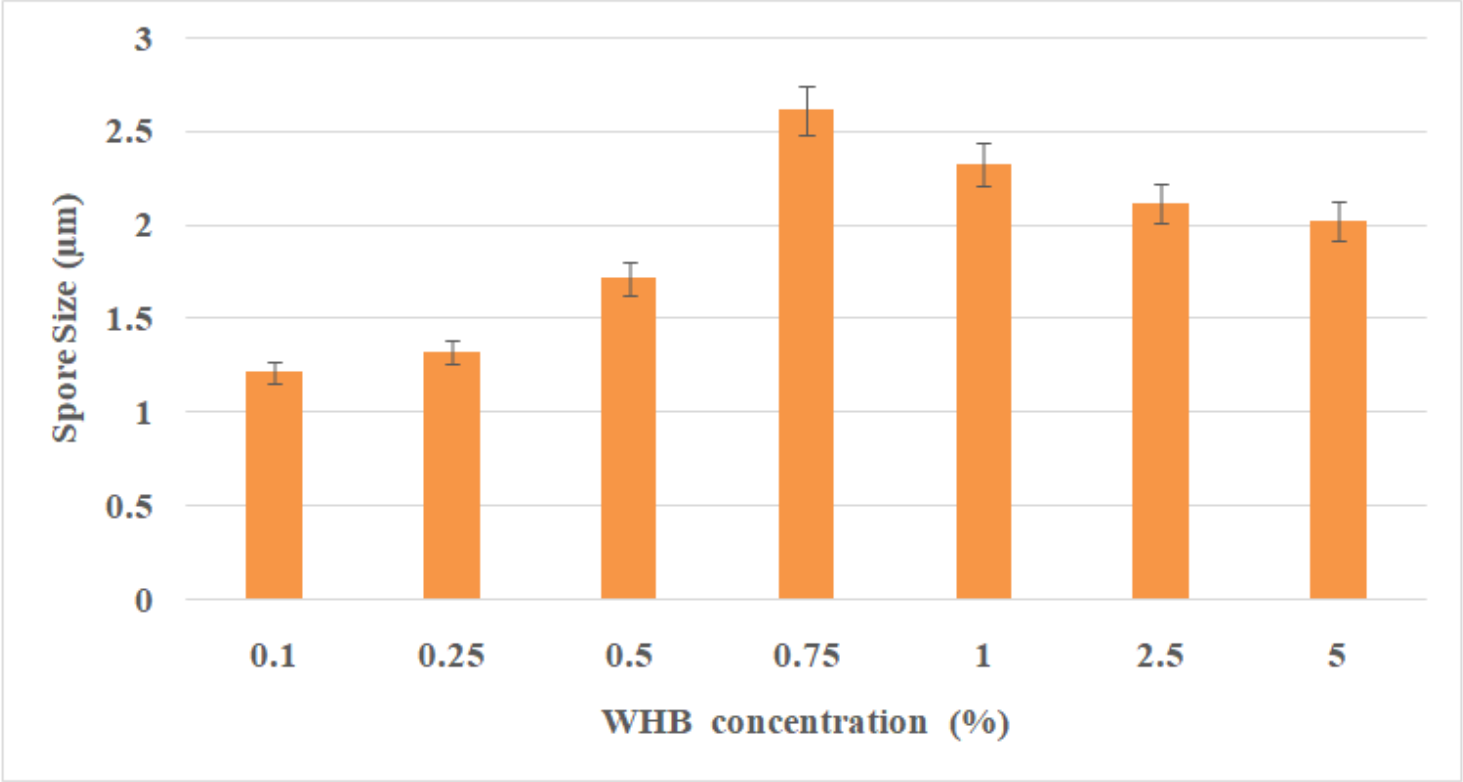

Figure 23

Effect of WHB media (%) on spore size of B.bassiana

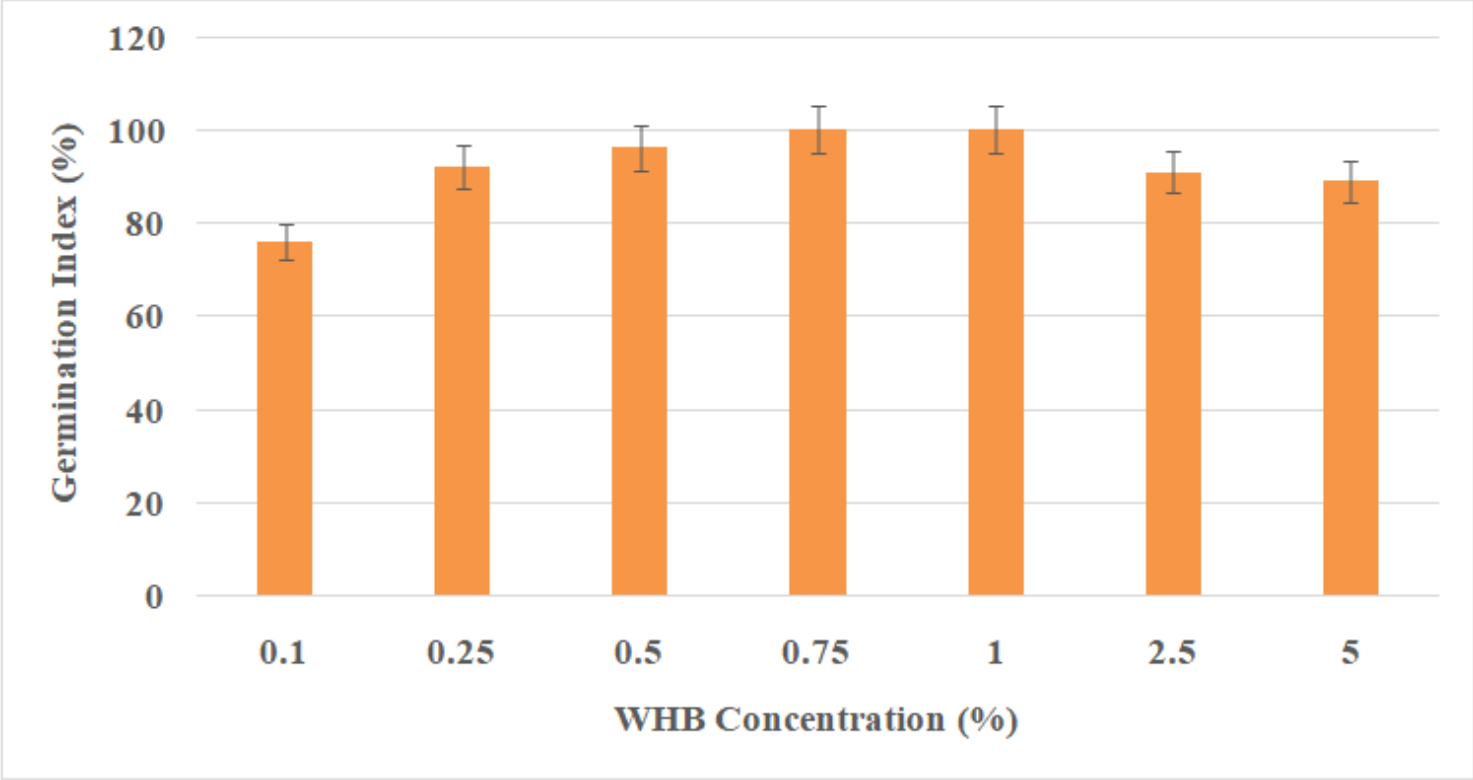

Figure 24

Effect of WHB medium on germination index of B.bassiana

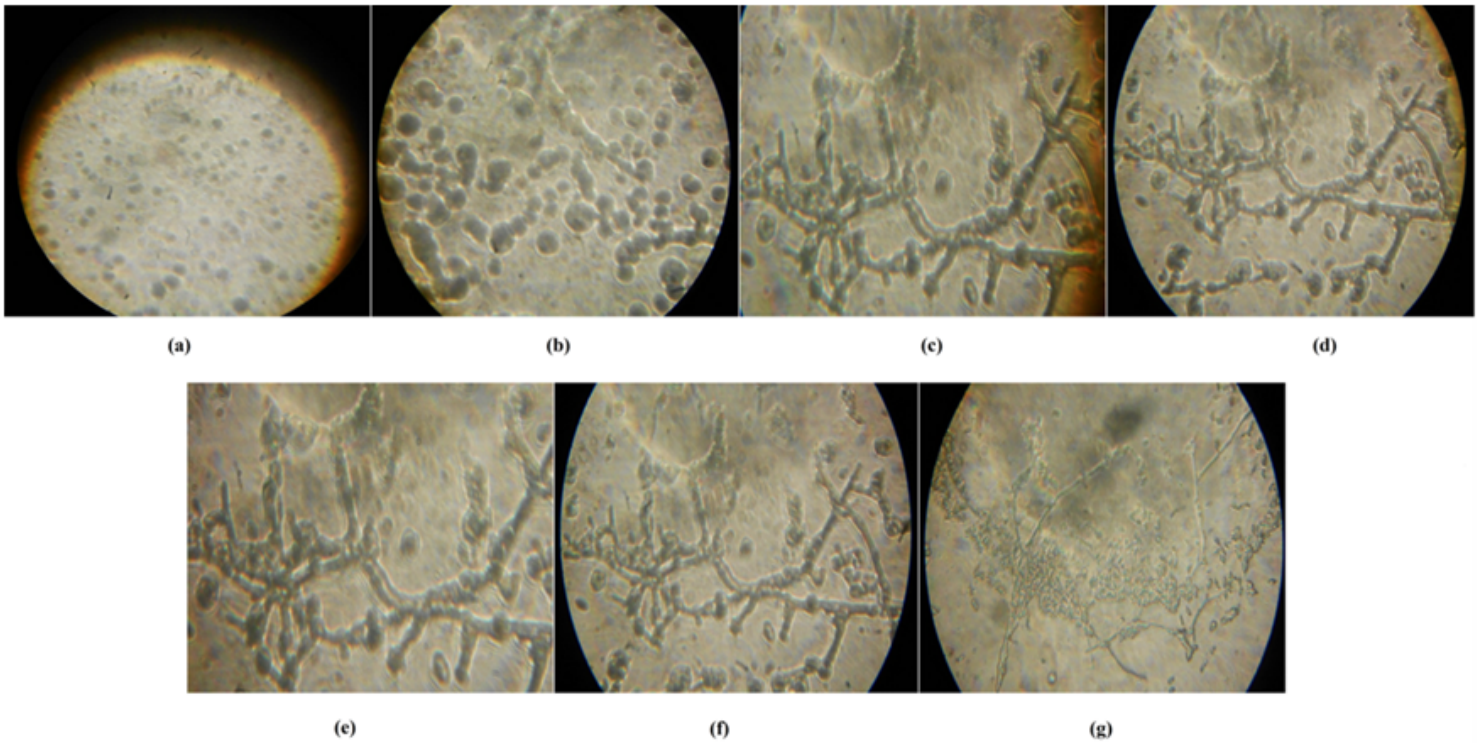

Figure 25

Microscopic examination of germinated spores of Beauveriabassiana derived from WHB media (a) 0.1 % (b) 0.25 % (c) 0.5 % (d) 0.75 % (e) 1.0 % (f) 2.5 % (g) 5.0 %

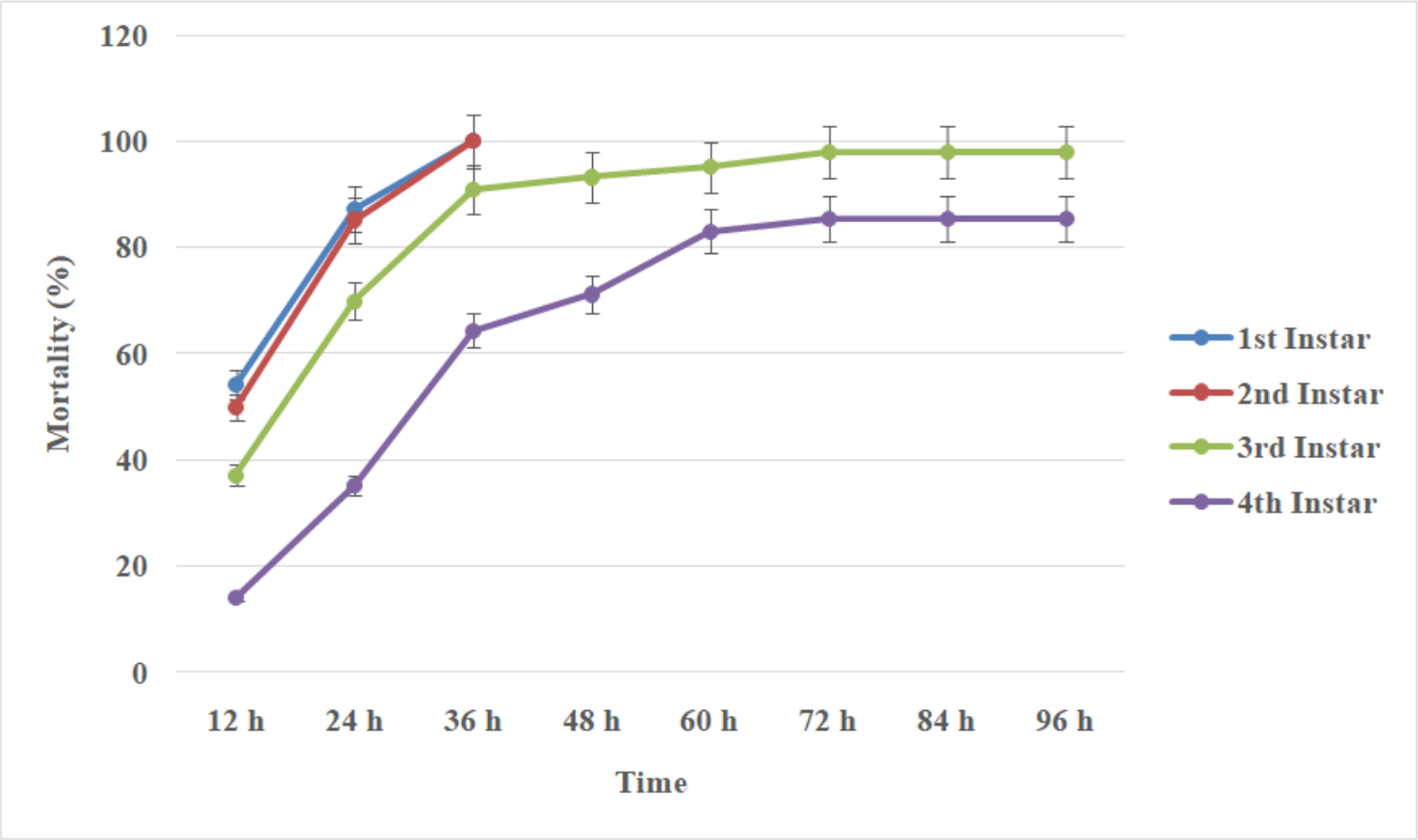

Figure 26

Effect of B.bassiana with 10 8 spores/ml dosage on mortality of Achaea janata

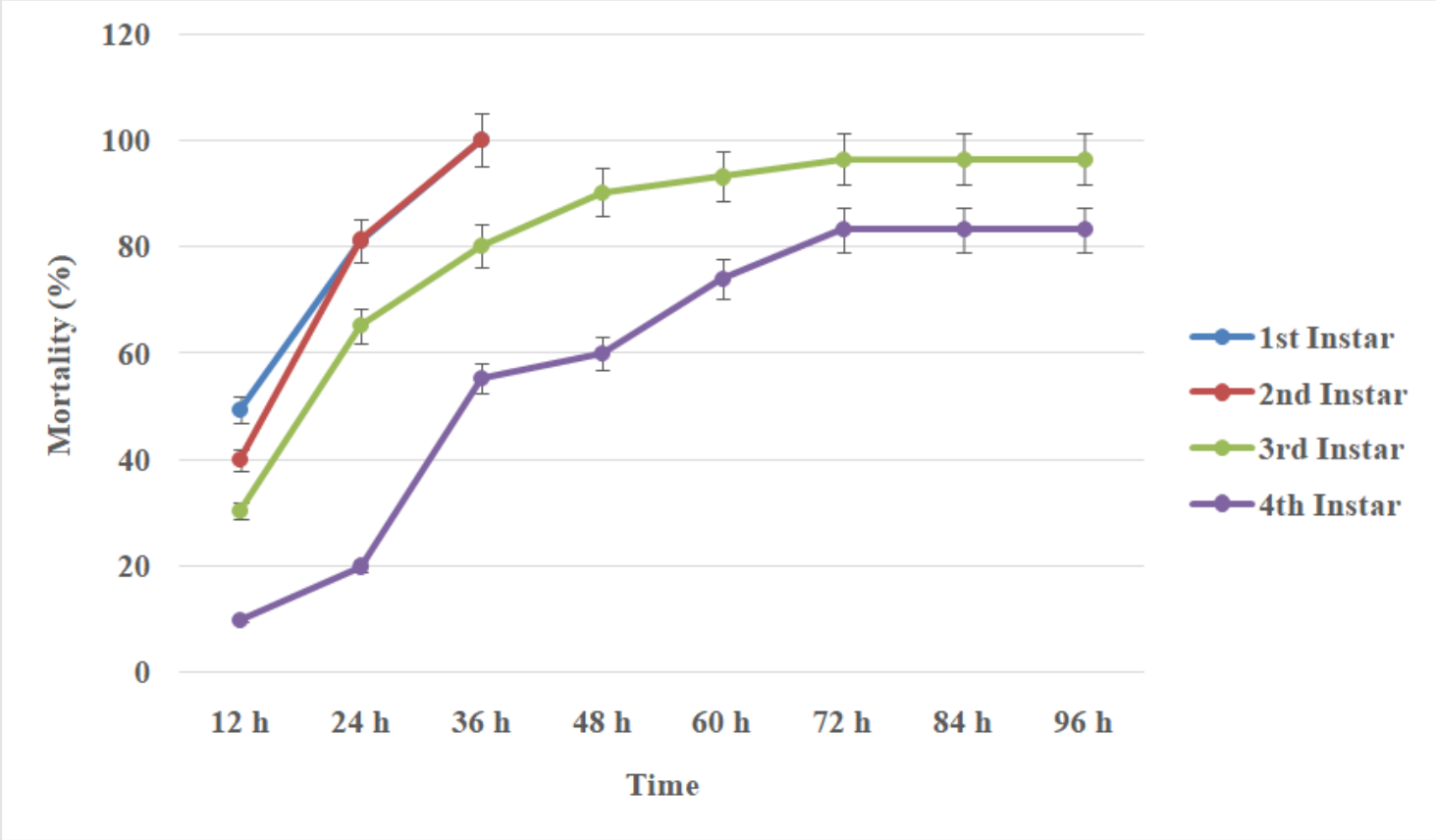

Figure 27

Effect of B.bassiana with 10 7 spores/ml dosage on mortality of Achaea janata

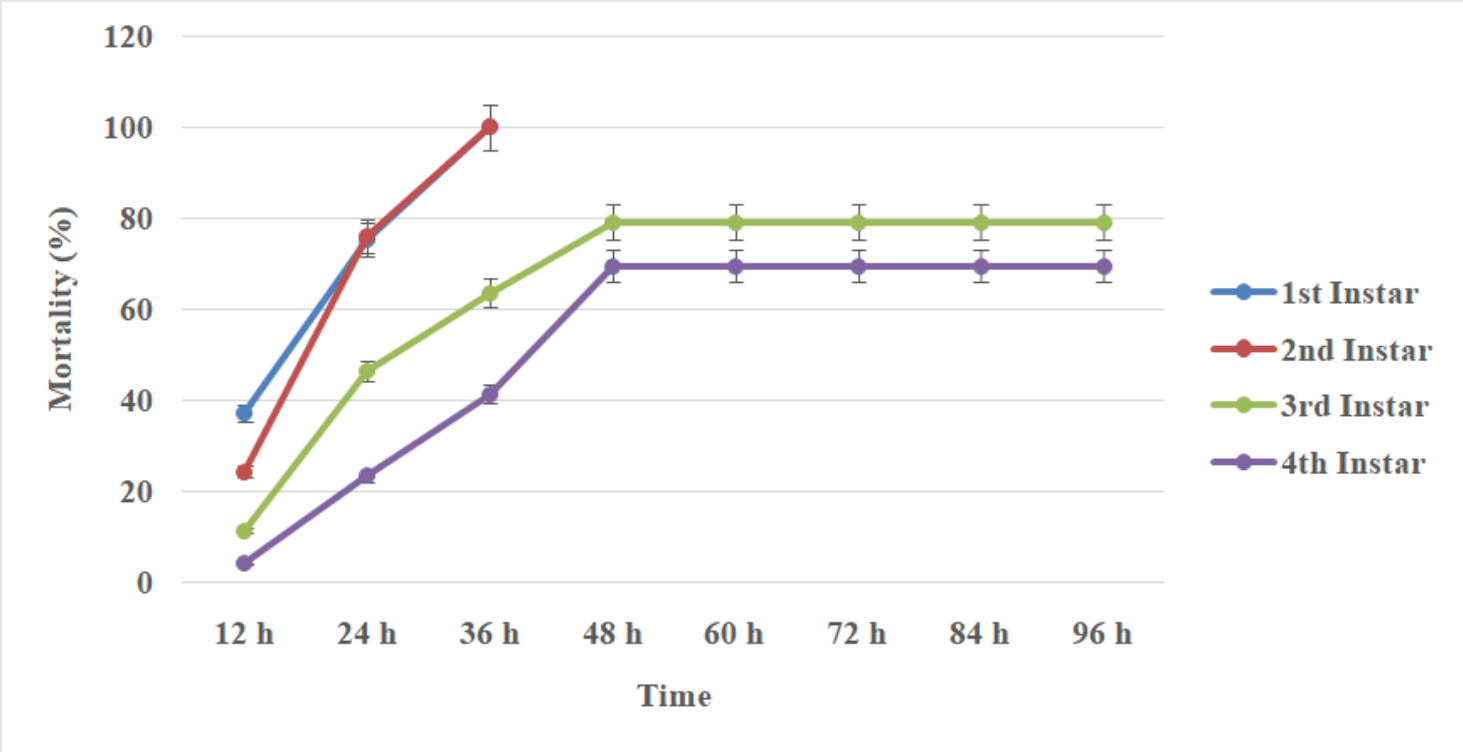

Figure 28

Effect of B.bassiana with 10 6 spores/ml dosage on mortality of Achaea janata

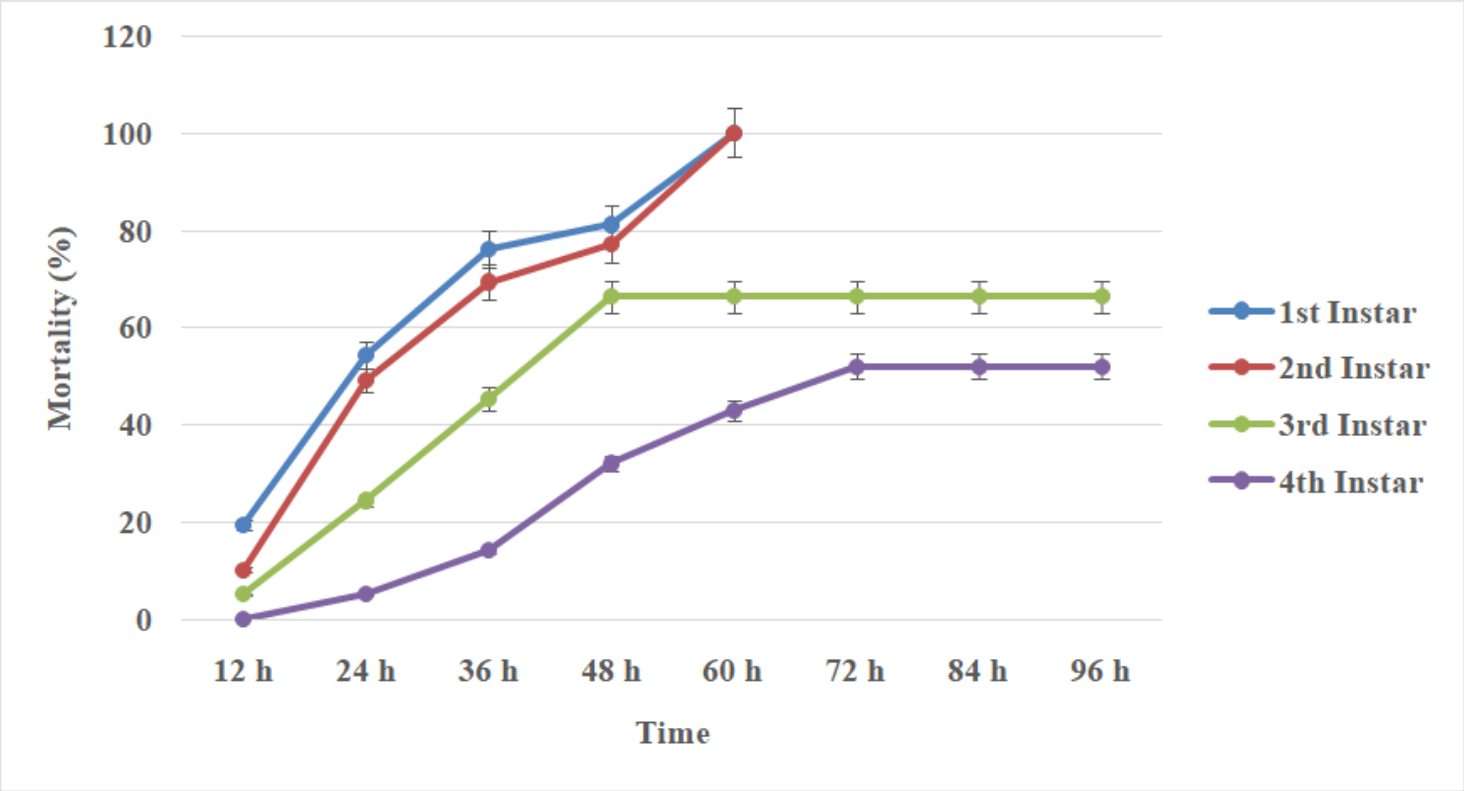

Figure 29

Effect of B.bassiana with 10 5 spores/ml dosage on mortality of Achaea janata

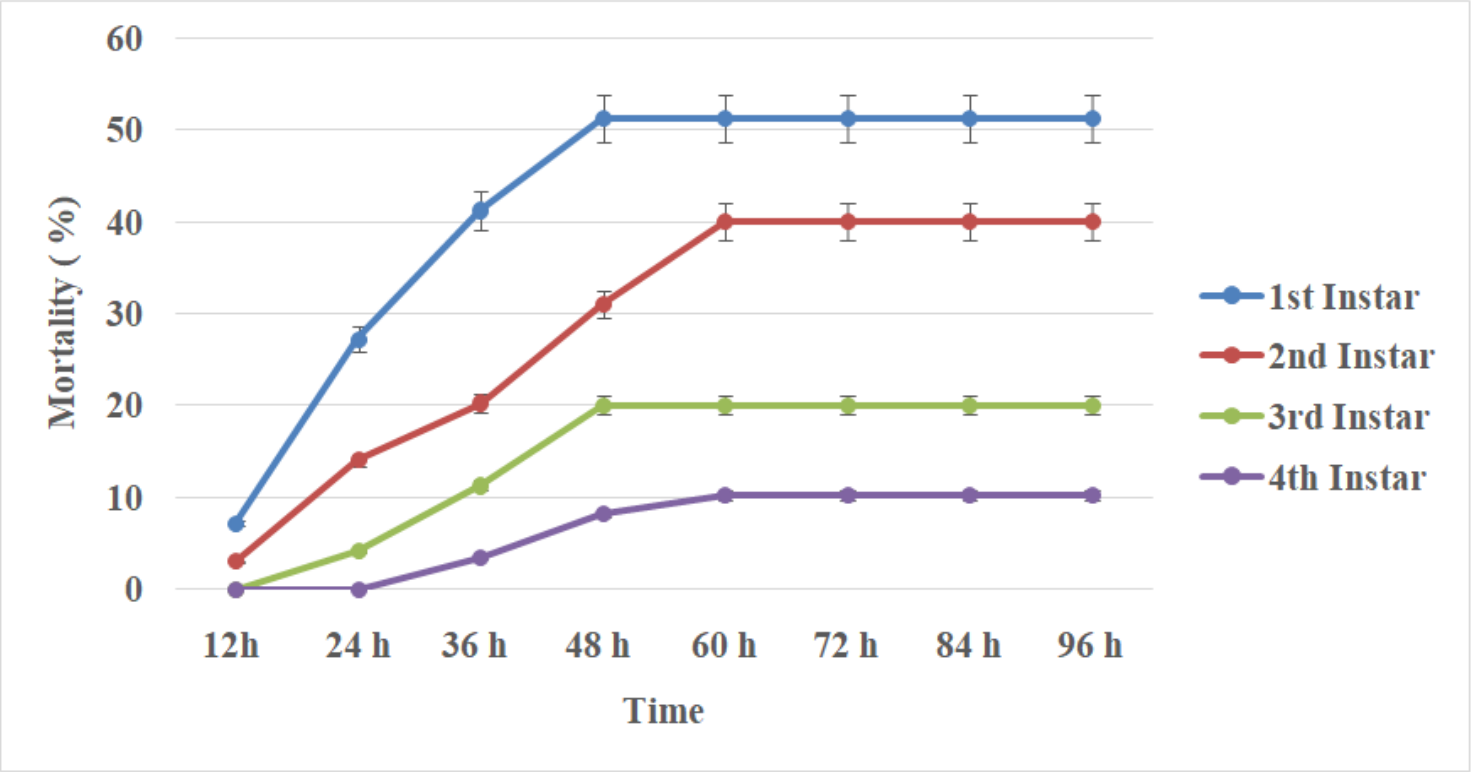

Figure 30

Effect of B.bassiana with 10 4 spores/ml dosage on mortality of Achaea janata