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Research Article

STUDY ON PHYSICOCHEMICAL PARAMETERS OF RAW SPENT WASH & ITS EFFECT ON SEED GERMINATION AND SOIL MICROFLORA

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Abstract: The main aim of the study was to see the effect of different concentration of raw spent wash on seed germination of selected crops and also its effect on soil micro-flora. The crops chick pea & lentil were selected for pot study to check their germination with different concentration of raw spent wash. Four treatments were taken 20% (Spent wash and water), 50% (Spent wash and water) and 100% (Pure Spent wash) along with control (100% water). Pots were irrigated with selected treatments at regular intervals to maintain moisture. Data revealed that increased concentrations of raw spent wash *i.e*., 50% and 100% completely inhibited the growth of both the crops, no germination was seen. At 20% concentration 13% germination was observed in case of lentil and 26% in chick pea as compared to control where 100% germination was observed in both the crops. Total viable count (cfu/ml) decreased with increased concentration of raw spent wash. It was also observed visually that white threads like structure were grown in 50% & 100% concentration on both set of soil samples. Effect of soil microflora was also seen continuously for 7days. The results indicated that raw and treated spent wash decreased the population of bacteria and fungi. Initial days *Aspergillus niger, Aspergillus fumigates, Penicillium, Mucor, Rhizopus, Aspergillus terreus, fusarium oxysporium, trichoderma* these were the common fungus seen in case of all the treatments and in both raw sets. But in latter in 50% & 100% concentration of Spent wash both sets only few species were predominant Aspergillus spp., Penicillium fusarium oxysporium and Mucor their hyphae covered the whole pot and inhibited the seed germination. The increased availability of soft carbon (reducing sugar and protein) and mineral in spent wash possibly favored the microbial growth initially, but later the bacterial count decreased only few dominated fungal colony were seen as network of Hypae also the seed placed for germination in pot was badly rotten in 50% &100% concentration of both sets.

Keywords: *Spent wash, Distillery, Nutrient, Micro-flora, Germination and fungi*

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Introduction

The aqueous distillery effluent stream referred to as spent wash is a dark brown highly organic cum acidic effluent and is approximately 10-15 times by volume of product alcohol [1, 2]. The disposal of distillery spent wash is of great concern because of its large volume and high biological oxygen demand (BOD) and chemical oxygen demand (COD). Analysis of distillery waste on physicochemical characteristics has been carried out, [3- 5] which stated that molasses is the most common raw material used in distilleries for bio-ethanol production. After alcohol distillation, huge volume of darkish coloured spent wash remains in the stills which is the most difficult waste products to dispose of, because of low pH and dark brown colour. Due to high concentration of organic load, distillery spent wash could be a promising source of renewable energy.

The effluent doesn't contain any toxic heavy metals because it is the waste from plant materials. It contains high amount of nutrients like nitrogen, phosphorous, potassium, sulphur and an outsized number of micronutrients. Recently, the presence of appreciable amounts of plant growth promoters' *viz*., gibberellin (GA) and indole ethanoic acid (IAA) have also been detected from spent wash which further increases the nutritional value of spent wash [6- 8]. The high concentration of calcium (Ca) (2050 – 7000 mg/l) in spent wash might need the power to reclaim the sodic soils kind of like that of gypsum because it also contains the calcium. The results might be ascribed to the nutrients and also the growth promoters like GA and IAA present within the spent wash [Table-1]. Among the plant nutrients, potassium (K) is found in higher amounts followed by nitrogen (N) and phosphorus (P). There have been also numerous studies done on impact of distillery effluent on soil and water quality [9-13]. Several drawbacks in using waste water for agriculture were determined.

Many reporters reported the problem of soil salinity, interaction of chemical constituents of the wastes with the uptake of nutrients. And some also reported changes in micro flora and soil property in the agricultural field irrigated with the wastewater. Diluted spent wash increased the expansion of shoot length, leaf number per plant, leaf area and chlorophyll content of peas. Few workers have also reported an adverse effect of different concentration of various industrial effluents on various crops.

One of the foremost important environmental problems faced by the planet is of management of wastes. Different industries create a range of waste water pollutants; which are difficult and expensive to treat. Waste water pollutants and their characteristics levels of vary significantly from one industry to the other. The employment of commercial waste as soil amendment has generated interest in recent time. The waste water produced from distillery continuously could cater the wants of irrigated crops. Keeping these factors in sight the current study has been formulated. The main aim of the study was to see the effect of different concentration of raw spent wash on selected crop and also its effect on soil micro-flora.

Materials and Methods

Collection of spent wash

An untreated (Raw) spent wash was collected from the distillery situated at Dhampur. Sterilized bottle was used for collecting spent wash & was capped tightly. Spent wash collected was analyzed for pH, TDS, COD, BOD and TSS and were also screened for the isolation of potential bacterial & fungal stains initially.

Experimental set up for spent wash application

Four treatments *viz*., 20% (Spent wash and water), 50% (Spent wash and water) and 100% (Purely Spent wash) along with control (100% water) were taken for the study of raw spent wash. The pots of 1 kg capacity were filled with soil and sand in 2:1 ratio. Seeds of Chick pea & lentil were sown in pots. Pots were irrigated with selected treatments at regular intervals to maintain moisture. Each treatment was replicated thrice.

Table-1 Important properties of spent wash are shown in the following table

SN	Parameters	Range
1	pH	3.9-4.3
2	EC (dS/m)	30.5-45.2
3	Biological Oxygen demand	46100-96000
4	Chemical oxygen demand	104000-134400
5	Total dissolved solids	79000-87990
6	Nitrogen	1660-4200
7	Phosphorous	225-3038
8	Potassium	9600-17475
9	Calcium	2050-7000
10	Magnesium	1715-2100
11	Sodium	492-670
12	Sulphate	3240-3425
13	Chloride	7238-42096
14	Zinc	3.5-10.4
15	Copper	0.4-2.1
16	Manganese	4.6-5.1
17	Gibberellic acid	3246-4943
18	Indole acetic acid	25-61

**All values in in mg/L unless otherwise given above*

Observation recorded

Germination and rhizospheric soil of different treatment were collected daily for seven days to study the soil microflora (Bacteria & fungi) from all the pots.

Isolation of bacterial strain

Initially soil samples used to fill the pots were taken for isolation of microbial strains. One gram of soil which was thoroughly mixed before filling the pot was taken in a test tube containing 9 ml of sterile distilled water. By serial dilution, the sample was diluted to 10-1, 10-2, 10-3,10-4,10-5.....10n times. 10-5 for times diluted samples was taken and plated in petri-plate containing Nutrient Agar Media (NAM) by pour plate technique. The inoculated plates were incubated at 37˚C for 24-48 hours. The same was repeated every day till 7th day. Soil from three replicate of each sample were taken and pooled for isolation of bacteria.

Physiological and Biochemical Tests

Methods of Somasegaran and Hoben [14] and Josey *et al*., [15] were used to identify the bacteria. The physiological and biochemical tests were conducted as described by Cappuccino and Sherman [16].

Isolation of Fungi

Initially soil samples used to fill the pots were taken for isolation of microbial strains. One gram of soil which was thoroughly mixed before filling the pot was taken in a test tube containing 9 ml of sterile distilled water. By serial dilution, the sample was diluted to 10-1,10-2,10-3,10-4,10-5.....10n times. 10-5 for times diluted samples was taken and plated in petriplate containing Potato Dextrose Agar Media (PDA) by pour plate technique. The inoculated plates were incubated at 28˚C for 24-48 hours. The plates were incubated at 25 + 20C for five days and fungi appearing on the medium were mounted over a clean slide, stained with lacto phenol cotton blue and observed under the microscope. The same was repeated every day till 7th day of experimental set up. Soil from three replicate of each sample were taken and pooled for isolation of fungi. The fungi were identified by using standard manuals such as Manual of soil fungi Gillman, [17].

Results and discussion

Germination - A pot culture experiment was conducted to evaluate the effect of different treatments of distillery spent wash, *i.e*., raw spent wash mixed with different concentration *viz*., 20%, 50 % and 100% on germination of lentil and chick pea. The data presented in [Table-2 & 3] revealed that increased concentrations of raw spent wash *i.e*., 50% and 100% completely inhibited the growth of both the crops, no germination was seen at these concentrations. The effect of this raw spent wash on the seed germination of lentil and chickpea showed that lower concentration that is 20% did not inhibit seed germination completely but only 13% germination was observed in case of lentil and 26% in chick pea as compared to control where 100% germination was observed in both the crops. The application of raw distillery effluent at a concentration of more than 20% had an inhibitory effect on seed germination under laboratory conditions. Similar results were reported in the case of rice, sorghum, cowpea, wheat, soybean and pea [18]. Though Ramana *et al*. [19] reported no inhibitory effect of spent wash on the seed germination of various vegetable crops, the seeds failed to germinate at higher concentrations. Therefore, specific care is required before the use of spent wash for irrigation purposes. The application of treated spent wash at higher concentrations resulted in poor development and establishment of plants. Other authors also reported that higher concentrations of effluent/spent wash retarded the rate of germination and seedling growth in soil Sahai *et al*., [18]. A high content of cations, anions and total dissolved solids has been reported to retard seed germination by enriching the salinity and conductivity of the solutes absorbed by the seed prior to germination Sahai *et al*., [18]. Total viable count (cfu/ml).

Table-2 Germination Index of lentil seeds treated with different concentration of raw

Lentil	Control	20%	50%	100%
		Conc of RSW	Conc of RSW	Conc of RSW
R1	100%	10%	NIL	NIL
R2	100%	20%	NIL	NIL
R3	100%	20%	NIL	NIL
Mean	100%	13%	Nil	Nil

Table-3 Germination Index of chick pea seeds treated with different concentration of raw spent wash (RSW)

Chick Pea	Control	20%	50%	100%
		Conc of RSW	Conc of RSW	Conc of RSW
R1	100%	30%	NIL	NIL
R2	100%	20%	NIL	NIL
R3	100%	30%	NIL	NIL
Mean	100%	26%	Nil	Nil

A pot culture experiment was conducted to evaluate the effect of different treatments of distillery spent wash, *i.e*., raw spent wash mixed with different concentration *viz*., 20%, 50 % and 100% on populations of bacteria and fungi. The results indicated that raw spent wash decreased the population of bacteria, and fungi. This study indicates that raw distillery wastewater was very toxic to the soil microorganisms which are important in the soil ecosystem. As observed from [Table-4] that total viable count (cfu/ml) decreased with increased concentration of raw spent wash. It was observed visually that white threads like structure were obtained in 100% concentration on both set of soil samples. On microscopic studies it shows the following structure showed dense network of thread like structure which appears blue in colour on staining. The bacterial count decreased with increased concentration of raw spent wash. A reduction in number of nodules per plant has been reported in various legumes after the application of distillery waste water (Juwarkar *et al*., 1990; Ramana *et al*., 2002b; Bhalerao *et al*., 2004). Because the toxic components of spent wash might have affected the population of Rhizobium and other micro-flora.

Visual observation

A pot culture experiment was conducted to evaluate the effect of different treatments of distillery spent wash, *i.e*., raw spent wash mixed with different concentration *viz*., 20%, 50 % and 100% on populations of bacteria and fungi. The data was observed right from the first day of application of raw spent wash. The soil samples from pots were collected from different concentration and after being serially diluted in sterile distilled water were plated on nutrient agar plates and then incubated for 48 hrs at 300C. Discrete bacterial colonies that grew on agar plates were initially grouped on the basis of gram staining & different morphology characteristics such as pigmentation motility & colony forms.

Table-4 Total viable count (cfu/ml) of soil micro-flora treated with different concentration of raw spent wash

Lentil							
Sample	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Control A	$8.1X10^{-4}$ cfu/ml	$5.3X10^{-4}$ cfu/ml	$5.1X10^{-3}$ cfu/ml	$6.2X10^{-3}$ cfu/ml	$7.8X10^{-3}$ cfu/ml	$5.1X10^{-3}$ cfu/ml	$7.2X10^{-3}$ cfu/ml
20%	$8.9X10^{2}$cfu/ml	$4.2X10^{-2}$ cfu/ml	$3.7X10^{-2}$ cfu/ml	$4.3X10^{-2}$ cfu/ml	$3.4X10^{-2}$ cfu/ml	$1.1X10^{-2}$ cfu/ml	$1.0X10^{-2}$ cfu/ml
50%	$7.1X10^{-2}$ cfu/ml	$2.1X10^{-2}$ cfu/ml	$1.9X10^{-2}$ cfu/ml	$2.4\ X10^{-2}$ cfu/ml	Spotted lawn	Spotted lawn	Spotted lawn
100%	$4.4X10^{-2}$ cfu/ml	Spotted lawn	Spotted lawn	Spotted lawn	Spotted lawn	Spotted lawn	Spotted lawn
Chick Pea							
Control B	$9.5X10^{-3}$ cfu/ml	$8.7X10^{-3}$ cfu/ml	$8.2X10^{-3}$ cfu/ml	$6.8X10^{-3}$ cfu/ml	$7.1X10^{-3}$ cfu/ml	$6.7X10^{3}$cfu/ml	$7.2X10^{-3}$ cfu/ml
20%	$7.8X10^{-2}$cfu/ml	$8.1X10^{-2}$ cfu/ml	$7.6X10^{-2}$ cfu/ml	$5.8X10^{-2}$ cfu/ml	$2.9X10^{-2}$ cfu/ml	$3.4X10^{-2}$ cfu/ml	$1.5X10^{-2}$ cfu/ml
50%	$6.7X10^{-2}$ cfu/ml	$2.2X10^{-2}$ cfu/ml	$2.0X10^{-2}$ cfu/ml	$1.3X10^{-2}$ cfu/ml	Spotted lawn	Spotted lawn	Spotted lawn
100%	$7.9X10^{-2}$ cfu/ml	Spotted lawn	Spotted lawn	Spotted lawn	Spotted lawn	Spotted lawn	Spotted lawn

Table-5 Macroscopic and microscopic visual characters of soil sample treated with different concentration of raw spent wash on 1st Day

Raw Spent wash							
SN	Sample	Macroscopic Characters			Microscopic characters		
	(lentil)	Colour	Shape	Elevation	Appearance	Shape	Gram stain
1	Control	Off white	Round	Flat	Rough	Rods	Negative
2	20%	Off white	Irregular	Flat	Rough	Cocci	Negative
3	50%	Off white	Irregular	Flat	Smooth	Rods	Negative
4	100%	Off white	Round	Flat	Rough	Cocci	Positive

Table-6 Macroscopic and microscopic visual characters of soil sample treated with different concentration of raw spent wash on 2nd Day

Raw Spent wash							
SNo	Sample	Macroscopic Characters			Microscopic characters		
	(lentil)	Colour	Shape	Elevation	Appearance	Shape	Gram stain
1	Control	Off white	Round	Flat	Rough	Rods	Negative
2	20%	Off white	Irregular	Flat	Rough	Cocci	Negative
3	50%	Off white	Irregular	Flat	Smooth	Rods	Negative
4	100%	Off white	Round	Flat	Rough	Cocci	Positive

Table-7 Macroscopic and microscopic visual characters of soil sample treated with different concentration of raw spent wash on 3rdDay

Raw Spent wash							
SNo	Sample	Macroscopic Characters			Microscopic Characters		
	(lentil)	Colour	Shape	Elevation	Appearance	Shape	Gram stain
1	Control	Off white	Round	Flat	Rough	Rods	Negative
2	20%	Off white	Irregular	Flat	Rough	Cocci	Negative
3	50%	Off white	Irregular	Flat	smooth	Rods	Negative
4	100%	Off white	Round	Flat	Rough	Cocci	Positive

Table-8 Macroscopic and microscopic visual characters of soil sample treated with different concentration of raw spent wash on 4th Day

Raw Spent wash							
SNo	Sample	Macroscopic Characters			Microscopic Characters		
	Chick Pea	Colour	Shape	Elevation	Appearance	Shape	Gram stain
1	Control	Off white	Round	Flat	Rough	Rods	Negative
2	20%	Off white	Irregular	Flat	Rough	Cocci	Negative
3	50%	Off white	Irregular	Flat	smooth	Rods	Negative
4	100%	Off white	Round	Flat	Rough	Cocci	Positive

Table-9 Macroscopic and microscopic visual characters of soil sample treated with different concentration of raw spent wash on 5th Day

Raw Spent wash							
SNo	Sample	Macroscopic Characters			Microscopic Characters		
	Chick Pea	Colour	Shape	Elevation	Appearance	Shape	Gram stain
1	Control	Off white	Round	Flat	Rough	Rods	Negative
2	20%	Off white	Irregular	Flat	Rough	Cocci	Negative
3	50%	Off white	Irregular	Flat	smooth	Rods	Negative
4	100%	Off white	Round	Flat	Rough	Cocci	Positive

Table-10 Macroscopic and microscopic visual characters of soil sample treated with different concentration of raw spent wash on 6th Day

Raw spent wash							
SNo	Sample	Macroscopic Characters			Microscopic Characters		
	Chick Pea	Colour	Shape	Elevation	Appearance	Shape	Gram stain
1	Control	Off white	Round	Flat	Rough	Rods	Negative
2	20%	Off white	Irregular	Flat	Rough	Cocci	Negative
3	50%	Off white	Irregular	Flat	smooth	Rods	Negative
4	100%	Off white	Round	Flat	Rough	Cocci	Positive

Table-11 Macroscopic and microscopic visual characters of soil sample treated with different concentration of raw spent wash on 7th Day

Raw spent wash							
SNo	Sample	Macroscopic Characters			Microscopic Characters		
	(lentil)	Colour	Shape	Elevation	Appearance	Shape	Gram stain
1	Control	Off white	Round	Flat	Rough	Rods	Positive
2	20%	Off white	Irregular	Flat	Rough	Cocci	Negative
3	50%	Off white	Irregular	Flat	smooth	Rods	Positive
4	100%	Off white	Round	Flat	Rough	Cocci	Positive

Table-12 Micro-organism found in soil before the application of raw spent wash on 1st Day

Bacteria	Fungi
Azotobacter	*Aspergillus*
Bacillus	*Penicillium*
Micrococcus	*Rhizopus*
P. vulgaris	
P. aeruginosa	

Table-13 Micro-organisms found after application of raw spent wash on 2nd Day

Lentil			
Control	20%	50%	100%
Bacillus	*Bacillus*	*Bacillus*	*Bacillus*
Micrococcus	*Azotobacter*	*Rhizopus*	*Aspergillus*
P. vulgaris	*Alternaria*	*Aspergillus*	*Rhizopus*
Rhizopus	*Aspergillus*		
Aspergillus			

Chickpea			
Control	20%	50%	100%
Acetobacter	*E.coli*	*Bacillus sp.*	*Bacillus*
Bacillus	*Bacillus*	*P. vulgaris*	*Aspergillus*
E.coli	*Aspergillus*	*Rhizopus*	*Rhizopus*
Aspergillus	*Alternaria*	*Mucor*	*Mucor*
Mucor			

Table-14 Micro-organisms found after application of raw spent wash on 3rd DAY

Lentil			
Control	20%	50%	100%
Bacillus	*Bacillus*	*Bacillus*	*Bacillus*
Micrococcus	*P. vulgaris*	*Acetobacter*	*Aspergillus*
P. vulgaris	*Mucor*	*Mucor*	*Rhizopus*
Rhizopus	*Rhizopus*		
Aspergillus			

Chickpea			
Control	20%	50%	100%
Bacillus	*Bacillus*	*Aspergillus*	*Bacillus*
P. aeruginosa	*Rhizopus*	*unknown*	*Aspergillus*
Penicillium	*Aspergillus*		
Rhizopus			

Bacteria isolates were then picked, sub cultured & subjected to further biochemical test for identification. But it was observed that majority of bacterial colony were off white in colour and only few were pure white. Few off white coloured colonies changed into orange colour flat round mucoid type of colonies after 48 hours of incubation.

The increased availability of soft carbon (reducing sugar and protein) and mineral in spent wash possibly favored the microbial growth initially, but later the bacterial count decreased only few dominated fungal colonies were seen as network of Hypae also the seed placed for germination in pot was badly rotten in 50% &100% concentration of both treated and raw spent wash treatment.

The results of investigation revealed that the raw spent wash contained different types of microscopic and macroscopic elements till 7 days after adding spent wash. Though the count reduced drastically as the concentration of raw spent wash increased but whatever few colonies grown were visually observed and present in [Table-5-11].

Identification of Bacterial Strains

Soil sample being serially diluted in distilled water were placed on nutrient agar plates & then incubated for 48 hrs at 30°C discrete bacterial colonies that grew on agar plates were initially grouped on the basis of gram staining & different morphology characteristics such as pigmentation motility & colony forms. Bacteria isolates were then picked, sub cultured & subjected to further biochemical test for identification according to Bergey's manual of determination bacteriology (9th edition) & then confirmed with help of PIB computer kit [Table-12-18]. Bacteria found was same as in earlier day, some results do not match with the bacterial result. Mainly *Azotobacter, Bacillus, Micrococcus, P Straita, Rhizobium* etc were found but their count decreased with increased in concentration of RSW.

Table-15 Micro-organisms found after application of raw spent wash on 4th DAY

Lentil			
Control	20%	50%	100%
Bacillus	*Bacillus*	*Bacillus*	*Bacillus*
Micrococcus	*P. vulgaris*	*Acetobacter*	*Aspergillus*
P. vulgaris	*Mucor*	*Mucor*	*Rhizopus*
Rhizopus	*Rhizopus*		
Aspergillus			

Chickpea			
Control	20%	50%	100%
Bacillus	*Bacillus*	*Aspergillus*	*Bacillus*
P. aeruginosa	*Rhizopus*	*unknown*	*Aspergillus*
Penicillium	*Aspergillus*	*Mucor*	*Orange fungi (unknown)*
Rhizopus			

Table-16 Micro-organisms found after application of raw spent wash on 5th DAY

Lentil			
Control	20%	50%	100%
Bacillus	*Bacillus*	*Bacillus*	*Bacillus*
Micrococcus	*P. vulgaris*	*Acetobacter*	*Aspergillus*
P. vulgaris	*Mucor*	*Mucor*	*Rhizopus*
Rhizopus	*Rhizopus*		*Mucor*
Aspergillus			

Chickpea			
Control	20%	50%	100%
Bacillus	*Bacillus*	*Aspergillus*	*Bacillus*
P. aeruginosa	*Rhizopus*	*unknown*	*Aspergillus*
Penicillium	*Aspergillus*	*Mucor*	*Mucor*
Rhizopus			

Table-17 Micro-organisms found after application of raw spent wash on 6th DAY

Lentil			
Control	20%	50%	100%
Bacillus	*Bacillus*	*Bacillus*	*Bacillus*
Micrococcus	*Azotobacter*	*P. aeruginosa*	*Aspergillus*
P. vulgaris	*Alternaria*	*Rhizopus*	*Mucor*
Rhizopus	*Aspergillus*	*Mucor*	
Aspergillus			

Chickpea			
Control	20%	50%	100%
Bacillus	*Bacillus*	*Bacillus*	*Bacillus*
Micrococcus	*Acetobacter*	*P. aeruginosa*	*Aspergillus*
P. vulgaris	*Alternaria*	*Rhizopus*	*Mucor*
Rhizopus	*Aspergillus*	*Mucor*	
A.niger, Rhizopus, Penicillium, Mucor			

Table-18 Micro-organisms found after application of raw spent wash on 7th DAY

Lentil			
Control	20%	50%	100%
Bacillus	*P. putida*	*P. vulgaris*	*Bacillus*
Micrococcus	*Azotobacter*	*Aspergillus*	*Mucor*
Azotobacter	*Alternaria*	*Mucor*	*Aspergillus*
P. putida	*Aspergillus*		
A.niger, Rhizopus Penicillium Mucor			

Chickpea			
Control	20%	50%	100%
Bacillus	*P. putida*	*P. vulgaris*	*Bacillus*
Micrococcus	*Azotobacter*	*Aspergillus*	*Mucor*
Azotobacter	*Alternaria*	*Mucor*	*Aspergillus*
P. putida	*Aspergillus*		
A.niger, Rhizopus Penicillium Mucor			

Table-19 Macroscopic and Microscopic characters of fungal strains

SNo	Macroscopic features	Microscopic features	Fungi
1	Black in color.	Large dark brown conidial heads.	*Aspergillus niger*
	Powdery texture.	Conidiophores are smooth-walled and hyaline.	
	Elevated growth.	Conidia are globose to sub globose, dark brown to black and rough walled.	
2	Blue-Green surface pigmentation with suede -like surface.	Dense felt of conidiophores with conidia.	*Aspergillus fumigates*
	Wrinkled growth.	Conidial heads are typically columnar, uniseriate.	
	Become black in color after ageing	green in color and rough walled	
3	Growth rate moderately rapid to rapid	Hyphae septate, hyaline	*Penicillium*
	Texture velvety to powdery green, white, yellow on the surface	conidiophores simple or branched	
4	rapidly growing fungus	Broad hyphae which scarcely or non-septate	*Mucor*
	new growth is white in color but turns a grayish –brown with aging	sporangiophores are long, may be branched & terminate in round spore filled sporangia	
	reverse plate a is pale white		
5	rapidly growing fungus	non septate or sparely septate broad hyphae	*Rhizopus*
	growth generally whitish in color which can turn brown with age	sporangiophores are brown in color & usually unbranched	
		they can be solitary or form clusters	
6	-growth rate is rapid	hyphae are septate and hyaline	*Aspergillus terreus*
	texture of colonies varies from downy to powdery	conidial heads are compact, columnar and biseriate	
	colony colour is beige to buff to cinnamon	conidia are globose to ellipsoidal	
	reverse is pale yellow to brown with yellow soluble pigments	conidiophore is hyaline and smooth walled	

Identification of Fungal Strains

The fungal evaluation and identification were done in the selected soil samples treated with different concentration of raw spent wash. It was observed that different types fungal colony was seen in different soil sample treated with different concentration of spent wash. For identification fungal culture was mounted on clean slides and stained with lactophenol cotton blue stain. The slides were observed under the microscope. The fungal strains were identified based on colony characteristics and staining methods [Table-19]. The mycofloristic composition of effluent sample varied significantly. It was observed that different types of fungal colony were seen in different soil sample treated with different concentration of spent wash. For identification fungal culture was mounted on clean slides and stained with lactophenol cotton blue stain. The slides were observed under the microscope.

Though different varieties of fungus were seen in control samples which reduced as the concentration of raw spent wash increased. In Initial days *Aspergillus niger, Aspergillus fumigates, Penicillium, Mucor, Rhizopus, Aspergillus terreus, fusarium oxysporium, trichoderma* these were the common fungus seen in case of all the treatments. But in latter in 50% & 100% concentration of spent wash only few species were predominant *Aspergillus* spp., *Penicillium fusarium, oxysporium* and Mucor their hyphae covered the whole pot [Table-12-18].

Conclusion

From the experimental observations it may be concluded that distillery spent wash when used at 20% v/v concentration gave very less germination means more dilution is required to get higher germination. It has also been observed that at higher concentration the fungal growth predominates which inhibits the seed germination and disturbs the micro-flora. Moreover, for distillery it is ZLD (Zero liquid discharge) therefore, it is advisable to abide the guidelines/ statuary rules for the application of raw spent wash always.

Application of research: The disposal of distillery spent wash is of great concern because of its large volume and high biological oxygen demand (BOD) and chemical oxygen demand (COD). Use of spent wash in agriculture before application need to be optimized or diluted as it may affects the germination of the crops. It may also bring the changes in soil micro flora.

Research Category: Effluent Management

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