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Collection Of Dhatura Seeds (Datura Metel Linn.) W.r.t. Microbial Load an Experimental Study.

KEYWORDS: Dhatura, Microbial load, Belgaum.

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ABSTRACT *Fresh plants are exposed to potential microbial contamination from farm to point of consumption. Bacteriological safety survey of Dhatura seeds (Datura metel Linn.) was carried out to assess the effect of selected antibacterial agents on their decontamination. The seeds were analyzed bacteriologically by total fungal count and total bacterial count. Seeds of Dhatura (Datura metel Linn.) were collected from Belgaum Railway Station, Karnataka, India. Results shows that before shodhan (purification) and after shodhan bacterial and fungal count is TNTC. Ayurveda highlights that medicinal plants should be collected keeping in view the appropriate habitat (Desha-sampat), appropriate season (Kala-sampat), and their effective attributes (Guna-sampat). One should collect the various parts of these plants while facing towards the east or north after performing auspicious rites/ rituals in a spirit of compassion, while living a pure life, while wearing white dress, after offering prayers to the Gods, Ashwinis, Cows and Brahmins, and while observing fast.*

Introduction:

It is often important to know not only what types of bacteria are in a sample but also how many of them are present. Food manufacturers are required by the FDA to monitor the number and type of bacteria in their products. Dairies monitor the number of bacteria present in milk after pasteurization. Water treatment plants monitor the effectiveness of their sterilization process. Very few of the things we eat or drink are bacteria free. They merely have greatly reduced numbers of "harmless" bacteria. Biotechnology firms closely regulate bacterial growth as they manipulate these organisms to produce useful pharmaceutical products. Beer and wine companies monitor the growth of yeast in their distilling process. Clinical laboratories monitor the growth rate of bacteria from patients to determine their antimicrobial sensitivity.[1] Total number of living microorganisms in a given volume or mass of microbiological media or food is called as microbial load.[2] Total Bacterial Count (TBC), also known as Heterotrophic Plate Count (HPC), Heterotrophic Colony Count (HCC), Aerobic Plate Count (APC), Total Plate Count (TPC), or Standard Plate Count (SPC), represents the total bacterial load in a given sample. It is a test to detect all viable microorganisms that could grow aerobically on plate count agar at appropriate incubation condition (usually 37°C, 48hrs). The TBC tests could reflect the general hygiene condition of a sample. Guideline values of TBC depend on nature of samples, and they may be varied in different countries. The following shows some of the guideline values of TBC.[3]

Total Population Count - Direct Methods:

Total population counts can be done manually, through direct observation of cells in a specialized counting chamber slide viewed under phase contrast microscopy. They can also be done electronically with a machine, such as a Coulter Counter that registers each cell as it passes through a small orifice. Values from these methods are reported as total bacteria per milliliter. Some of the problems associated with these techniques are that bacteria are very small and hard too see even with phase contrast. Bacteria often clump or string together. The actual bacterial numbers can be higher than what is reported because the clumped bacteria get counted as one big bacterium. These methods also count all the bacteria present in the sample, dead or alive. These counts, however, are quickly done.[4]

Viable Counts:

It is often necessary to determine how many live bacteria are actually in a sample, especially when measuring growth rates or determining disinfectant effectiveness. This involves the serial dilution of bacteria samples and plating them on suitable growth media. You can also filter your samples through a membrane which you place on a pad soaked with growth media. The plates are incubated until you see visible colonies, usually 18-24 hours. The colonies you see growing on the plate are considered to have started from one viable bacterial unit. Because bacteria are usually not found as individuals, the colony you see may have started from a single cell or a group of cells. The results are reported as colony forming units (CFU's).[5]

Plate Count Procedure:

There are several methods commonly used for plate counting bacteria: pour plate, overlay plate, and surface count. For the pour and overlay method the bacterial sample is suspended in molten agar that is just barely warm enough to keep the agar from setting up. It is then poured into an empty Petri dish or poured in a thin layer on another agar surface. The advantages of these methods are that the colonies stay small and compact. You can count plates with a lot higher concentration because the colonies will not be touching one another. The main disadvantage is the difficulty in keeping the agar hot enough to keep it from setting up until you pour it and cool enough to not heat shock or kill you bacteria. The surface count plate method gives reliable and consistent results. It is far easier to use. You pipette a small volume of bacteria onto the surface of a plate and spread it evenly around the surface. It is also useful if you are using selective media because you do not obtain the same color responses when the bacteria are growing in the agar because of the different oxygen re-

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quirements. Before you do any of this however, you need to dilute down any bacterial culture that has visible turbidity. Refer to the Serial Dilution Procedure for this.[6]

Determining a Viable Count for an Unknown Culture Using OD Values

Use Microsoft Excel on the computers to make a table and graph of your data.

1. Make a scatter graph.

Use the y-axis, non-log, for the absorbance data plotted in OD units (0-2). Remember that absorbance increases as the number of bacteria increases. On the x-axis plot the dilution factors: 1, 0.5 (1:2), 0.25 (1:4), 0.125 (1:8) and 0.0625.

2. Add a trendline to your scatter graph.

3. Since you know the number of CFU/mL from your plate count serial dilutions, you should be able to calculate the number of viable CFU from a different culture grown under the same conditions using this graph.

4. Obtain an OD value for your new culture.

5. Use the standard curve you just made to determine where the OD value of your new culture falls on the trend line you plotted.

Match this OD value up to the corresponding dilution factor, then multiple that by the CFU/mL of your standard. This gives you the CFU's/mL of your new culture.[7]

MATERIALS AND METHODS:

Study area:

Belgaum is nestling high in Western Ghat in Karnataka, India. It lies in zone of cultural transition between Karnataka, Maharashtra and Goa. Seeds of Dhatura (Datura metel Linn.) was collected from Belgaum Railway station. Latitude is 15.7333, Lat (DMS) is 15° 43′ 60N, Longitude is 74.3833, Long (DMS) is 74° 22′ 60E, Altitude (feet) is 2516, and Altitude (meters) is 766 above sea level.[8]

Sample preparation:

Prepared 90 ml Soybean Casein Digest Media (HiMedia; Lot No: 0000139150). Weigh 10 grams/- ml of sample into 90 ml Soybean Casein Digest Media in the sterile safety cabinet. Mixed the sample properly to make it homogenous. Incubated the sample at 37^0 C for 24 hours. (Time: 12:35 p.m.), Date: 28/11/13.

1 ml of sample from Soybean Casein Digest Medium tube was added to two sterile empty plates. Poured approx. 20-25 ml of liquefied Sabourauds Dextrose Agar to the plate. (HiMedia; Lot No: 0000135208). Allowed the plates to stand for 15-20 minutes to get solidify. Incubated the plates in incubator in an inverted position at 30-35^0 C for 5 days. Time (12:58 p.m.). Date: 28/11/13.

Observation:

Plate no.	Day 3 (Time: 4:15 p.m.). Date: 2/12/13 Cfu/ml	Day 5 (Time: 10:30 a.m.) Date: 3/12/13 Cfu/ml	Day 5 (Time: 10:30 a.m.) Date: 3/12/13 Cfu/ml
1.	TNTC	TNTC	TNTC
2.	TNTC	TNTC	TNTC
			Total Count: TNTC Cfu/ml

RESULTS:

REPORT NO: CRF/MU/1035/13

Sample: Shodita Dhatura Beeja Churna
Date: 3/12/13

Product: Dhatura Seed
Date of Receipt: 28/11/13

Date: 28/11/2013
Part form: Seed

(* N/A: Not available)
Sample quantity: 15 grams

Description Macroscopic:

Sr.No.	Organisms	Limit (As per IP)	Result
1.	E. Coli	Absent	Absent
2.	S. Aureus	Absent	Absent
3. P. Aeruginosa Absent			Absent
4.	S. Abony	Absent	Absent

(Standards referred above are as per IP)

Description Macroscopic:

Sr.No.	Count	Limits	Results
5.	Total bacterial count	30-300 cfu/ml	TNTC
6.	Total fungal count	10-100 cfu/ml	TNTC

Description Macroscopic:

REPORT NO: CRF/MU/1035/13

Sample: Ashodita Dhatura Beeja Churna Date: 3/12/13

Product: Dhatura Seed Date of Receipt: 28/11/13

Date: 28/11/2013 Part form: Seed

(* N/A: Not available)
Sample quantity: 15 grams

Sr.No.	Organisms	Limit (As per IP)	Result
1.	E. Coli	Absent	Absent
2.	S. Aureus	Absent	Absent
3.	P. Aeruginosa	Absent	Absent
4.	S. Abony	Absent	Absent

(Standards referred above are as per IP)

Description Macroscopic:

Sr.No.	Count	Limits	Results
5.	Total bacterial count	30-300 cfu/ml	12 cfu/ ml
6.	Total fungal count	10-100 cfu/ml	TNTC

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Sample preparation:
Prepared 90 ml Soybean Casein Digest Media (HiMedia; Lot No: 0000139150). Weigh 10 grams/- ml of sample into 90 ml Soybean Casein Digest Media in the sterile safety cabinet. Mixed the sample properly to make it homogenous. Incubated the sample at 37^0 C for 24 hours. (Time: 12:35 p.m.), Date: 28/11/13.

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Observation:

Plate no.	Day 3 (Time: 4:15). Date: 2/12/13 Cfu/ml	Day 5 (Time: 10:30) Date: 3/12/13 Cfu/ml	Day 5 (Time: 10:30) Date: 3/12/13 Cfu/ml
1.	TNTC	TNTC	TNTC
2.	TNTC	TNTC	TNTC
			Total Count: TNTC Cfu/ ml

Image 1: Bacterial and Fungal Growth of Shodhit and Ashodhit Dhatura Seeds.

DISCUSSION AND CONCLUSION:
The majority of the Dhatura seeds (Datura mete*l* Linn.) in this study were grossly affected. The effects were attributed to poor source of collection. Drugs are required to be collected keeping in view the appropriate habitat (Desha-sampat), appropriate season (Kala-sampat), and their effective attributes (Guna-sampat). One should collect the various parts of these plants while facing towards the east or north after performing auspicious rites/ rituals in a spirit of compassion, while living a pure life, while wearing white dress, after offering prayers to the Gods, Ashwinis, Cows and Brahmins, and while observing fast.

Plants which are grow on ant hills, filthy ground, marshy places, burial ground, barren and saline soils and open streets infested with insects do not serve any medicinal purpose. Plants affected by fire or cold are useless. So one should take care while collection of parts of plants while using for medicinal purposes.

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