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Formulation of Eco-Friendly, Sustainable Herbal Disinfectant for Aviary (Bird) Shelter

Jeffrrin Lizzi. J.S

Student, Dr.N.G.P Arts And Science College

Abstract:
The term "Pet bird" refers birds housed and bred for an ornamental purpose. This includes mainly passeriformes (e.g. canaries, finches, sparrow) and psittaciformes (parrots, parakeets, budgerigars, love birds) [1]. These birds are potential carriers and or transmitters of zoonotic diseases. Some of these pathologies could have an important impact on human health, like chlamydophilosis, salmonellosis or even highly pathogenic avian influenza A H5N1. The microbial population in the aviary cage or shelter could be controlled by proper hygiene practices and preventive measures. Preventive measures include Proper disinfection of aviary cage. The one stop solution against the chemical disinfectants was Herbal formulation of Disinfectants .This project combines the usage of a single or combination of multiple Herbs. This study is based on the formulation of herbal disinfectant from the herbs Rosemary(Salvia rosemarinus), Thyme(Thymus vulgaris), Oregano(Oreganum vulgare). The inhibitory effect of rosemary is the result of the action of rosmarinic acid, rosmaridiphenol, carnosol, epirosmanol, carnosic acid, rosmanol and isorosmanol.

INTRODUCTION

The term "Pet bird" refers birds housed and bred for an ornamental purpose. This includes mainly *passeriformes* (e.g. canaries, finches, sparrow) and *psittaciformes* (parrots, parakeets, budgerigars, love birds) [1]. These birds are potential carriers and or transmitters of zoonotic diseases. Some of these pathologies could have an important impact on human health, like *chlamydophilosis, salmonellosis* or even highly pathogenic avian influenza A H5N1, but also have an economic impact if some of these pathogens are spread via carriers or vectors like wild birds, human beings, insects or mites to poultry breeding units or cattle facilities , then entering the food chain [2].

There are two main types of transmission routes they are 1) Direct transmission,

2) Vector borne transmission which includes Direct transmission- Households, Pet shops, bird fairs and markets. Vector borne transmission- Mites, Mosquitoes, Ticks. *Passeriforms and psittacines* are housed under different conditions, due to their respective behavior. Besides the "kitchen-housing", which is usually a single cage typically containing a couple of canaries or budgerigars for example, *passeriform* species are rather kept in captivity in two different types of aviaries mixed ornamental aviaries and breeding facilities.

The first type is usually a big wire-netting space (up to 10 m3) located outside and sometimes with different species kept together, mostly for ornamental purposes . In the second type, relatively large numbers of the same species, depending on the breeding size and breeding purpose (pet shops versus competitions) are maintained in pairs [2]. In direct relationship with local breeders, housing of birds in pet shop facilities enhances the risk of transfer of several zoonoses, like for example *chlamydophilosis*.

Cages are indeed often overcrowded, filled with birds of mixed origin [3].
The overcrowdingalso induces intense stress to the birds due to territory or food. This will cause quick debilitation of the weakest individuals and higher sensitivity to infections [4].
psittaci from birds-tohumans in France and The Netherlands in such conditions. In both cases, clinical symptoms were developed by patients and led in several cases to hospitalization [6]. Vector-borne diseases represent a major problem for public health. Bird ectoparasites, especially mesostigmatic mites belonging to Dermanyssidae and Macronyssidae, are well known for their heavy potential to transmit diseases to poultry. *Dermanyssus gallinae* in particular, even if exhaustively described in poultry breeding, is also an underestimated pet bird pathogen.
This mite is often found in both the pet bird family household and intensive breeding. *D. Gallinae* has been described to cause an important debilitation by exsanguination, involving high mortality rate in newborns, and sometimes in hens. *D. gallinae* has also been proven to transmit zoonotic pathogens, such as *C. psittaci, Coxiella burnetii, Salmonella spp, Erysipelothrix rhusiopathiae , Listeria monocytogenes* and viruses like Fowl pox virus [7],[8],[9]. Different species of mosquitoes (Diptera, especially Culex species) are responsible for horizontal and reciprocal transmission of arboviruses like West Nile fever Virus [10].
Dipterae act as bridging vectors between two host categories: amplificators (e.g. birds) and incidental/deadend (e.a. humans)[11]. Studies have shown that house sparrows develop WNV viremia superior to 10^{10} pfu/mL afterexperimental infection, and maintain it above 105 pfu/mL for five days [13],[14] and they are indeed good amplificatory hosts and, moreover overwintering hosts [12] for at least one arbovirus, the WNV [10] Ticks from the genus *Ixodes (e.g. I. ricinus, I. scapularis*), are carried by birds and then have the ability to transmit pathogens like Borrelia burgdoferi, the causative agent of Lyme disease, and the flavivirus louping illvirus. [15].
One of the most threatening zoonotic bacterial diseases transmitted by birds to humans is *chlamydophilosis* (also known as *chlamydiosis, ornithosis*, psittacosis or parrot fever), caused by the intracellular bacterium *Chlamydophila psittaci.* Human symptoms comefrom mild respiratory signs to severe pneumonia, with localization in several organs leading to diarrhea, conjunctivitis, arthritis and genital organ infection [16].
Highly pathogenic avian influenza A H5N1 has been in the world health focus since the year 2000's outbreaks. Perkins and Swayne demonstrated in 2003 that the avian influenza A virus H5N1 after intranasal administration was able to induce clinical symptoms leading to death in pet bird species like zebra finches and common budgerigars, which are very common hosts of domestic ornamental aviaries, as well as in wild species like house sparrows and European starlings, usually living close to human habitations **[17].**Pigeons are known to be reservoirs of pathogenic yeasts, like *Cryptococcus neoformans*, which is described to cause opportunistic infections in humans **[18].**
Several studies have demonstrated the presence of *C. neoformans* in parrots, littlepet birds like canaries, budgerigars or lovebirds and cockatiels **[19,20].** Despite a relatively poor documentation on pet bird parasitic diseases, *giardiosis, aspergillosis and cryptosporidiosis* have been reported in these avian populations, both in chronic and in acute infections. Favorizing conditions could be high-density populations, stress, and adaptation to a new environment or prolonged periods in confined housing **[21]**. Transmission to humans often results from feces manipulation or lack of hygiene. This can be prevented to some extent by disinfecting the aviary cage or shelter.

2. REVIEW OF LITERATURE

Health Hazards

Poultry and aviary farmers, are at increased risk of occupational respiratorydiseases Exposure to organic dust is one of the most recognized respiratory hazards associated with animal production.[22]. Dust, bacteria, moulds, endotoxin and ammonia are considered central elements in daily exposure of agricultural workers . These substances are known to cause allergic and non-allergic rhinitis, asthma, extrinsic alveolitis, organic dust toxic syndrome and can also induce chronic bronchitis[23]. Epidemiological studies showed increased prevalence of respiratory symptoms and adverse changes in pulmonary function parameters in these workers[24]. the exposure to moulds and house dust mite *Dermatophagoides pteronyssinus (Pyroglyphidae)* on two poultry farms.

Health effects of occupational exposure to organic dust in poultry workers, including allergic sensitization and the effects on respiratory system[25]. work environment of poultry houses involves variable exposure to respiratory andskin irritants, pro-inflammatory agents and allergens. Hazardous levels of endotoxin, main allergen of dust mite *Dermatophagoides pteronyysinus* and moulds (particularly *Aspergillus, Penicillium and Mucor* species) were determined. In line with these occupational exposures, significantly higher prevalence of work-related respiratory, eye and skin symptoms was found in poultry workers in comparison to control subjects. Poultry workers also had higher prevalence of IgG antibodies to moulds, especially *Alternaria and Aspergillus* species, and slight decline in ventilatory lung function[26].

Histoplasmosis is caused by a fungus (Histoplasma capsulatum) found primarily in the areas drained by the Mississippi and Ohio rivers. Both humans and animals can be affected. The disease is transmitted to humans by airborne fungus spores from soil contaminated by pigeon and starling droppings (as well as from the droppings of otherbirds and bats). The soil under a roost usually has to have been enriched by droppings for two years or more for the disease organism to reach significant levels.

Although almost always associated with soil, the fungus has been found in droppings (particularly from bats) alone, such as in an attic Infection occurs when spores, carried by the air are inhaled — especially after a roost has been disturbed. Most infections are mild and produce either no symptoms or a minor influenza- like illness. On occasion, the disease can cause high fever, blood abnormalities, pneumonia and even death. In some areas, including portions of Illinois, up to 80 percent of the population show evidence of previous infection. Outbreaks of histoplasmosis have occurred in Central Illinois.

The National Institutes of Health (NIH) has reported a potentially blinding eye condition — presumed ocular histoplasmosis syndrome (OHS) — that probably results from the fungus. NIH estimates that 4 percent of those exposed to the disease are at risk of developing OHS.

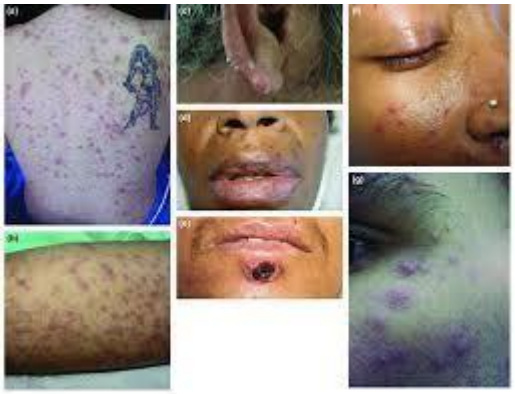


Figure No:1 Histoplasmosis

Pigeon droppings appear to be the most important source of the disease fungusCryptococcus neoformans in the environment. The fungus is typically found in accumulations of droppings around roosting and nesting sites, for example, attics, cupolas, ledges and water towers. It has been found in as many as 84 percent of samples taken from old roosts. Even when old and dry, bird droppings can be a significant source of infection.

Like histoplasmosis, most cryptococcosis infections are mild and may be without symptoms. Persons with weakened immune systems, however, are more susceptible toinfection. The disease is acquired by inhaling the yeast-like cells of the organism. Two forms of cryptococcosis occur in humans. The generalized form begins with a lung infection and spreads to other areas of the body, particularly the central nervous system, and is usually fatal unless treated. The cutaneous (skin) form is characterized by acne-like skin eruptions or ulcers with nodules just under the skin.

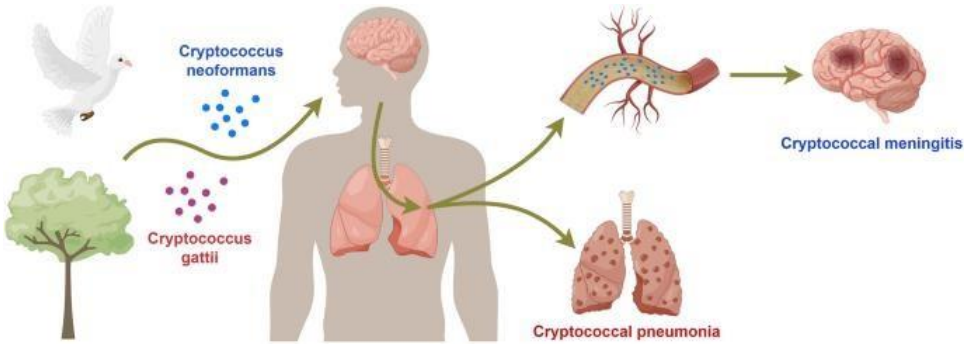


Figure No :2 Cryptococcal infection

The cutaneous form is very rare, however, without generalized (systemic) disease. Outbreaks (multiple cases at a location) of cryptococcosis infections have not been documented. Other diseases carried or transmitted by birds affect man to a lesser degree. Psittacosis is normally mild in man; however, serious illness can occur rarely. Pigeons and sparrows also have been implicated (along with many other species of birds) as reservoirs for encephalitis viruses such as West Nile encephalitis virus, which are carried by mosquitoes.

These are associated with a few diseases that affect people, such as rabies and histoplasmosis. Rabies is a dangerous, fatal disease, but only about 5 percent of bats submitted for testing are infected with the rabies virus. In recent years, there has been increased concern about the risk of rabies transmission following contact with bats. If aninjured or ill bat is found in or around a structure, it should be removed. Because most bats will try to bite when handled, they should be picked up with tongs or a shovel.

If has bitten or scratched a person or pet or is found in your home, capture the bat without touching it with your hands and without crushing its head. If the bat is dead, refrigerate it (DO NOT freeze) and then contact your local health departmentimmediately for instructions. Bats with rabies have been identified in most areas of the state. In recent years, bats have been the most common animal identified with rabies in the state. The incidence of histoplasmosis being transmitted from bat droppings to humans is not thought to be high.

Nevertheless, fresh bat droppings (unlike fresh bird dropping) can contain the histoplasmosis fungus. Bat droppings do not need to come into contact with soil to be a source of the disease

Bird roosts can harbor parasites that may invade buildings. Although these parasites can bite and irritate, they are unlikely to transmit diseases to humans. The northern fowl mite and chicken mite are usually the main culprits. Other parasites that may cause problems inside buildings include the pigeon nest bug and the bat bug (both related to the bed bug), soft ticks, biting lice and the pigeon fly. Although most

parasites associated with bird or bat roosts die quickly after the birds or bats leave, some may live for several weeks.

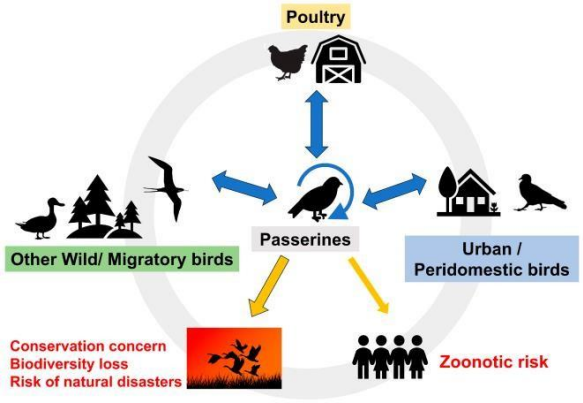


Figure No :3 Aviary Zoonosis Transmission

Economic Loss

The aviary diseases could lead to financial loss to both the breeder or the overall country's economy if the infection has spread across the city. For example HPAI- Highly pathogenic avian influenza. The movement of migratory birds has caused outbreaks to emerge in several countries and regions simultaneously, [27].

and global trade. The direct cost in loss of life and human productivity has not yet been large, although all deaths are regrettable and cause considerable distress to families involved. Considerably more has been spent on the costs of tracing disease and trying to prevent it.

The impact of a single outbreak of HPAI on national GDP depends on the speed with which it is controlled, the extent to which it spreads, the contribution of poultry to GDP and the structure of the poultry sector**.[28]** In the six most severely affected countries, the contribution of the poultry sector to GDP ranges from approximately 0.5% in Thailand to 1.3% in China and 1.5% in Cambodia. In Vietnam, before the final extent of spread was known, the costs of the 2003-4 outbreaks were predicted top be between 0.3%- 1.8% of GDP3 . Based on GDP estimates for 2004, this would have been the equivalent of US$76m450m. Earlyestimates in Thailand suggested that as much as 1.5% of GDP growth over a year was lost[29].

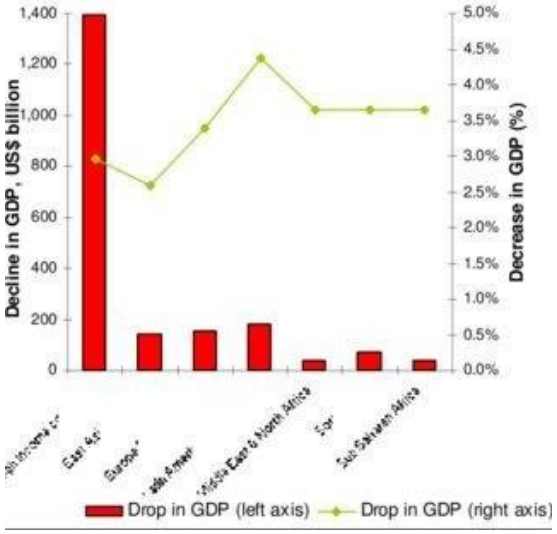


Figure No :4 Economic Impact of Avian Diseases

Control Measures

The microbial population in the aviary cage or shelter could be controlled by proper hygiene practices and preventive measures. Preventive measures include Proper disinfection of aviary cage.

Disinfection is the process of removing micro-organisms including pathogens from the inanimate objets, not necessarily killing the microorganisms but reducing to a acceptable level[30]. There are different levels of disinfection they are "High level disinfection" which indicates removal of all microorganisms."Intermediate level disinfection" which kills all the vegetative bacterias, but not against some pathogenic spores and virus.

"Low level disinfection" which kills vegetative bacteria, fungi and viruses. This could be chemical or herbal disinfectants. Chemical disinfectants comes with the negative impact[31]. Chemical disinfectant can be flammable vapour liquid.and canbe harmful if contacted with skin causing severe burns and irritation of eyes. , and damage of respiratory tract damage if prolonged or repeated exposure if inhaled, and toxic if swallowed and can be carcinogenic[32

Solutions

The one stop solution against the chemical disinfectants was Herbal formulation of Disinfectants .This project combines the usage of a single or combination ofmultiple Herbs. These cleaners are eco-friendly and do not harm the environment in the water that chemical cleaners do. They are free of harmful chemicals and are easier on the skin, means that you will no longer get cracked skin and blisters that you may have experienced after using chemical cleaners. These products are made with natural ingredients and hence, have natural and pleasant fragrances. This is in stark comparison to chemical cleaners which have the odour of bleach and chemicals whichreminiscent of hospitals and commercial spaces[33].

Herbal Formution

This study is based on the formulation of herbal disinfectant from the herbs Rosemary(*Salvia rosemarinus*), Thyme(*Thymus vulgaris*), Oregano(*Oreganumvulgare*). The inhibitory effect of rosemary is the result of the action of rosmarinic acid, rosmaridiphenol, carnosol, epirosmanol, carnosic acid, rosmanol and isorosmanol. They interact with the cell membrane, causing changes in genetic material and nutrients, altering the transport of electrons, leakage of cellular components and production changes in fatty acid.

In addition, it also produced an interaction with the membrane of proteins that produced the loss of membrane functionality and its structure [34]. Thyme consists of anti-microbial compound carvacrol which primarily relies on bacterial cell membrane damage, and thymol distrupts the permeability of the cytoplasmic membrane[35]. Oregano consists of Tannins explained by interference with the cell metabolism. And Flavonoids via various mechanisms by suppress nucleic acid synthesis, cytoplasmic membrane function, and metabolism[36].

Advantages

Using green or environmentally friendly items shields clients from exposure to chemicals. No longer will there be chemical concoctions absorbed into the skin or taken in by the individual cleaning. No irritated skin, eyes, a running nose, consistent coughing, asthma or other serious side effects related to destructive and harmful chemical substances. Traditional cleaning items are typically costly. This is a direct result of the various chemical compounds utilised in making them. Green cleaning items like

lemon vinaigrette, baking soda, and others are more affordable strong chemical smell that can frequently trigger migraines for those who have a low resilience to strong-smelling substance.
Now and then it is hard to remain or work in such a space since it is hard to breathe in the majority of the occasions. Most green items sold in the market, or custom-made ones, utilises characteristic ingredients that radiate wonderful smell, for example, citrus, lavender, lemongrass, and so forth. Eco- friendly items, are good for the earth since they contain natural ingredients. Changing to greener techniques lessens contamination to our conduits and the air and it limits your effect on ozone depletion

3. MATERIALS AND METHODS

Study Design

The study design was a laboratory study and it was divided into three parts. The first part is isolation of Micro-organisms from the live aviary cage- *Fringillidae,* the second part is extraction of the herbal disinfectant from the selected herbs -*Salvia rosmarinus, Thymus vulgaris, Origanum vulgare,* and the final part is where thedisinfectant is subjected to several tests for testing the efficiency.

Sampling Techniques

The samples for isolation of micro-organisms from the live aviary shelter were collected using surface swabbing method [37]. Three swabs were collected from the live bird cage using sterilized swabs stick and transported to the laboratory using a screw cap tube sterilized using saline. For the second part the dried herbs - *Salvia rosmarinus, Thymus vulgaris, Origanum vulgare* were collected from the herbal store-NeutraVed and stored in a zip lock pouch.

Isolation of Microorganisms

The swab samples were inoculated in the selected media plates. For bacterial isolation the inoculation was made in two EMB (Eosin methylene blue agar), and two MacConkey plates, and incubated at 37^{o} Celsius for 24 hours. For Fungal isolation in SDA (Sabouraud Dextrose Agar) plate and Candida (HiChrome) media plates at room temperature 27^{o}-30^{0} celsius for 48-72 depending the growth level[38,39]. The bacterial plates were given for MALDI-TOF analysis.

Extraction of Herbs

The collected dry herbs were powdered and stored in a screw cap tube (figure 1) and the next proccess is Soxhlet for extraction of the metabolites. The powdered herbswas weighed for 5.60 grams *Salvia rosmarinus*-1.86 gms, *Thymus vulgaris*-1.86 gms, *Origanum vulgare*-1.86 gms (figure 2).
[40]. Soxhlet extaction was performed upto 5 cycles (figure 4) until the extract becomes colourless, after completion the extract was stored in the covered brown glass bottles (figure 5).

Figure No :5 (Storage of powdered herbs - Rosemary(*Salvia rosemarinus*), Thyme(*Thymus vulgaris*), Oregano(*Oreganumvulgare*)

Figure No :6 Weighing of the powdered herbs for soxhlet process

Figure No :7 Transfer of the powder in cellulose sheet for soxhlet extraction

Figure No :8 Soxhlet Extraction process

Figure No: 9 (Storage of the extract in the dark bottle to prevent the oxidation process)

MIC (Minimal Inhibitory Concentration)

MIC procedure is performed to detect the anti-microbial efficiency of the herbal extract against the selected Microorganisms. The **MIC** value is the lowest concentration of an antibiotic at which bacterial growth is completely inhibited. Three plates of Muller-Hinton agar was prepared. Each microorganisms were selected from Gram negative – *Escherichia coli* (figure 6), Gram positive – *Staphylococcus aureus* (figure 7), Fungi – *Candida albicans* (figure 8). The selected microorganisms were streaked in the respective media plates using continuous streak method and three wellswere made using agar well cutter and the extract were filled in the well in three different concentrations 30 µl,40 µl, 50 µl using sterile pipette and incubated at 37^{o} Celsius for bacterial plates and room temperature for fungal plates for 24 hours [41].

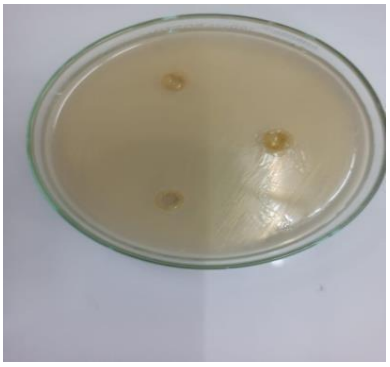


Figure No :10 (MIC plates inoculated with *Escherichia coli* on concentrations - 30 µl,40 µl, 50 µl)

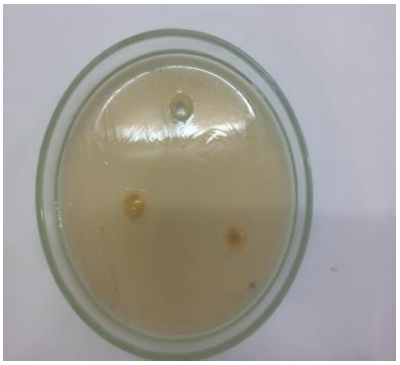


Figure No :11 (MIC plates inoculated with Staphylococcus aureus on concentrations - 30 µl,40 µl, 50 µl)

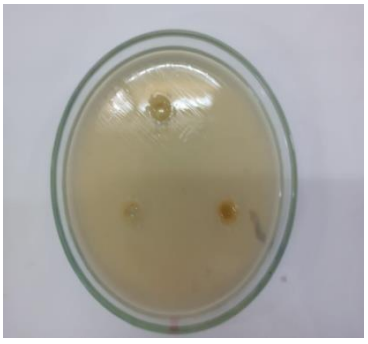


Figure No :12 (MIC plates inoculated with Candida albicans on concentrations - 30 µl,40 µl, 50 µl)

Phenol Coefficient Testing

Phenol coefficient test is performed to measure the disinfecting power or effectivenessof a disinfectant. The selected procdure was Rideal Walker method. The extract was tested against the standard phenol and the commercially brought Dettol. The process initiates with the preparation of stock solution (diluting 5ml of phenol in 100ml od distilled water)

And serially diluted into 1:100, 1:120, 1:140, 1:160 dilutions (figure 9,10). And the test disinfectant and dettol were made upto thedilution of 1:1000, 1:1200, 1:1400, 1:1600 dilutions (figure 11). The selected microorganisms were *Escherichia Coli, Pseudomons aureginosa, Staphylococcus aureus and Candida albicans* (figure:12,13,14,15). Nutrient agar plates were prepared for bacteria and SDA plates for Fungi. The selected microorganism were inoculated in the serially diluted test samples and inoculated into the plates after 2.5 mins, 5mins, 7.5 mins, 10mins and the plates were incubated at room temperature for 24 hours[42].

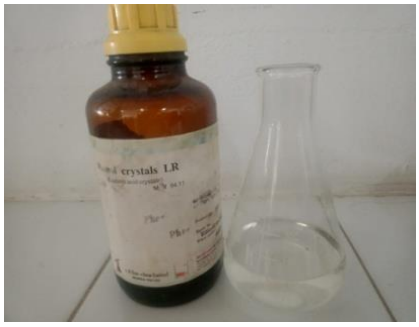


Figure No: 13 (Preperation of Phenol Stock Solution)

Figure No :14 (Dilution of Phenol Stock solution 1:1000 1:1200, 1:1400, 1:1600)

Figure No :15 (Dilution of Test Disinfectant, Dettol solution 1:100, 1:120, 1:140, 1:160)

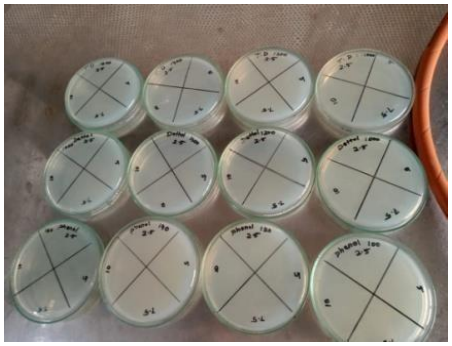
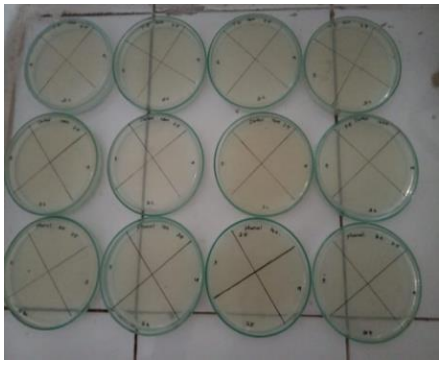


Figure No :16 (Inocuation of phenol, Test disinfectant,Stock Dettol, Phenol with *Escherichia coli*)



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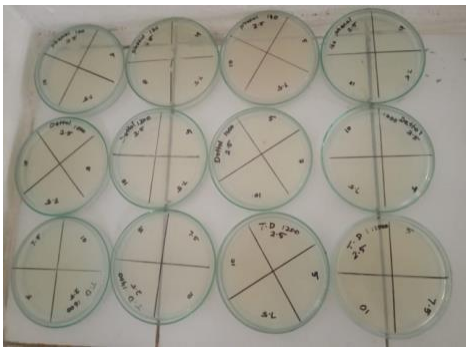

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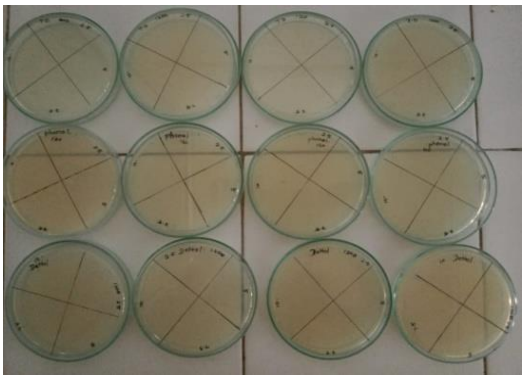


Figure No :19 (Inocuation of phenol, Test disinfectant,Dettol, Phenol with *Candida albicans*)

Live Cage Analysis

The test disinfectant was tested for its efficiency against microorganisms in the live aviary cage using the selected dilution 1:1000. The disinfectant was sprayed against the surface of the aviary cage for disinfection. The swab samples were collected from the surface Before disinfection and 2.5mins, 5mins, 7.5mins, 10mins after disinfectionand inoculated in the prepared Nutrient agar plates and SDA plates and incubated at room temperature for SDA plates and 37^0 Celsius for Nutrient agarplates for 24 hoursand the results were observed after 24 hours.

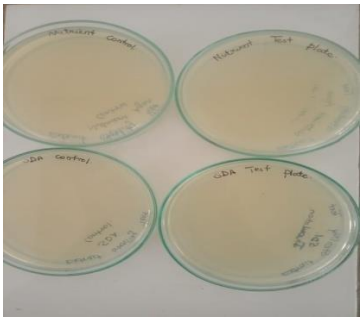


Figure No :20 (Inoculation of Swab collected from the live aviary shelter before and afterin time duration of 2.5mins. 5mins. 7.5mins, 10mins.)

Extract Sterlity Check

To conclude the extract has been subjected for sterlity check which is performed to analyze and ensure the absence of the viable microorganisms in the prepared herbal extract. This process initiates with the preparation of Two Nutrient agar media plates and two SDA agar plates each one for inoculation and for control plate. Inoculate the extract in the media plates using a sterile loop using continuous streaking procedure and incubate the plates at room temperature for 24 hours (Nutrient agar plates), 48 hours(SDA plates) and observe the results.

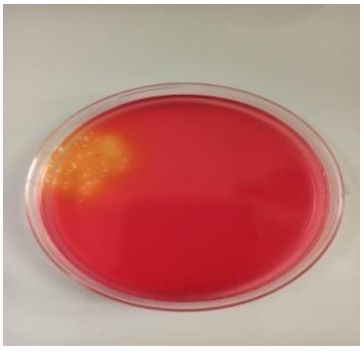
4. RESULTS

The results of the Microbial isolation, MIC (minimal inhibitory concentration), Phenolcoefficient test, Live cage analysis, and sterility check were presented below.

Isolation of Microorganisms

The incubated plates were observed for growth and colony appearance. The Mannitol salt agar plates shown golden yellow mucoid colony appearance(figure 17,18). The first MacConkey plate shown isolated pink mucoid colonies(figure 19) and in the second plate the colonies were mucoid, colourless colonies(figure 20) and in the HiChrome agar white mucoid colonies were seen(figure 21).

And the MALDI-TOF Analysis shows that the organism present in the Mannitol Sat agar plates were *Staphylococcus aureus*, and *Staphylococcus epidermis* . In Mac Conkey plates the organisms were *Escherichia coli* and *Pseudomonas aureginosa*.



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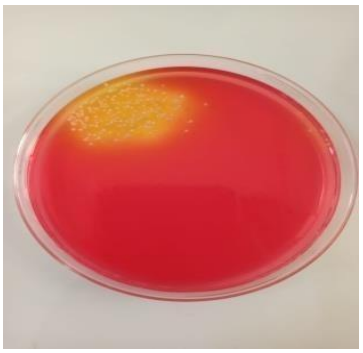

Figure No :22

Figure No :23

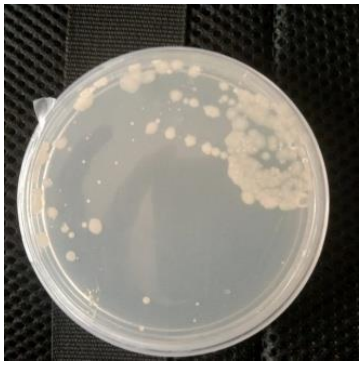

Figure No :24

The incubated plates were analysed for the Zone of inhibition. The size of zone ofinhibition indicates the antimicrobial efficiency of the extract against the selected microorganism .The larger zone of inhibition

usually means that the efficiency is morepotent. Table No :1 refers the results of the MIC test.

ORGANISM	CONCENTRATION		
	30 µL	40 µL	50 µL
Escherichia coli	5mm	7mm	9mm
Staphylococcus aureus	-	7mm	9mm
Candida albicans	-	-	8mm

Table No :1 (Results of Minimal inhibitory concentration test)

Phenol Coefficient Testing

The phenol coefficient test inoculated plates were observed for the microbial growth after 24 hours of incubation. And the growth was denoted by the + symbol and the inhibition of growth was denoted by – symbol. The following tables indicate the results of the results of *Escherichia coli* (Table no 2), *pseudomonas aeruginosa*(Tableno3), *Staphylococcus aureus* (Table no 4) and *Candida albicans* (Table no 5).

SAMPLE	DILUTION	DURATION			
		2.5mins	5mins	7.5mins	10mins
Phenol	1000	+	-	-	-
	1200	+	-	-	-
	1400	+	-	-	-
	1600	+	-	-	-
Test Disinfectant	100	+	+	-	-
	120	+	+	-	-
	140	+	+	+	-
	160	+	+	+	+
Dettol	100	+	+	-	-
	120	+	+	+	-
	140	+	+	+	+
	160	+	+	+	+

Table No :2 (Phenol coefficient results of *Escherichia coli*)

SAMPLE	DILUTION	DURATION			
		2.5mins	5mins	7.5mins	10mins
Phenol	1000	+	-	-	-
	1200	+	+	-	-
	1400	+	+	-	-
	1600	+	+	-	-
Test Disinfectant	100	+	+	-	-
	120	+	+	+	-
	140	+	+	-	-

	160	+	+	+	+
Dettol	100	+	-	-	-
	120	+	+	-	-
	140	+	+	+	-
	160	+	+	+	+

Table No :3 (Phenol coefficient results of *Pseudomonas aeruginosa*)

SAMPLE	DILUTION	DURATION			
		2.5mins	5mins	7.5mins	10mins
Phenol	1000	+	-	-	-
	1200	+	+	-	-
	1400	+	+	+	-
	1600	+	+	+	+
Test Disinfectant	100	+	+	-	-
	120	+	+	+	-
	140	+	+	+	-
	160	+	+	+	-
Dettol	100	+	+	-	-
	120	+	+	-	-
	140	+	+	+	-
	160	+	+	+	+

Table No :4 (Phenol coefficient results of *Staphylococcus aureus*)

SAMPLE	DILUTION	DURATION			
		2.5mins	5mins	7.5mins	10mins
Phenol	1000	+	-	-	-
	1200	+	+	-	-
	1400	+	+	+	-
	1600	+	+	+	-
Test Disinfectant	100	+	-	-	-
	120	+	+	-	-
	140	+	+	-	-
	160	+	+	+	-
Dettol	100	+	+	+	-
	120	+	+	-	-
	140	+	+	+	-
	160	+	+	+	-

Table No :5 (Phenol coefficient results of *Candida albicans*)

Live Cage Analysis

There is no growth observed in the control plates. Plate full of growth was observed in plates in which the samples collected before disinfection were inoculated as Nutrient agar plates and SDA plates. And the results after disinfection were analysed, the growth is observed in the inoculation only in 2.5mins, thus the timetaken to act against bacteria is 2.5mins(Figure 22). Growth in SDA plates were seen in 2.5mins, 5mins, time taken to act against fungi is 5mins. (Figure 23)

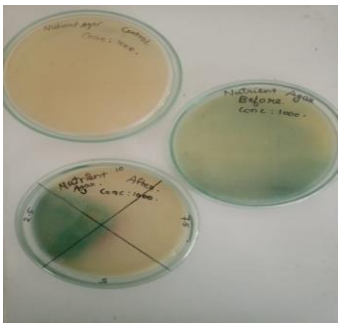

Figure No :25 (Bacterial growth on Nutrient agar plate in the inoculation of 2.5 mins.)

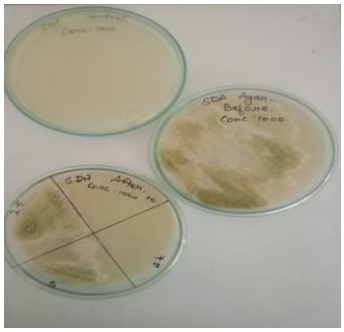

Figure No :26 (Fungal growth on SDA agar plates in the inoculation of 2.5 mins and 5 mins.)

Extract Sterlity Check

The plates inoculated with the extracted herbal disinfectant was observed for microbial growth after 24 hours, the presence of microbial colonies indicate the contamination of the extract. After interpretation the results were no growth is observed in the Nutrient media plates (figure 24) and SDA plates (figure 25) which indicate the sterility of the herbal extract.

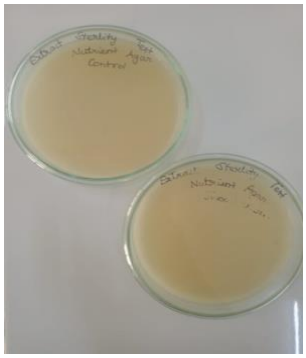

Figure No :26 (No growth observed in Nutrient agar plates)

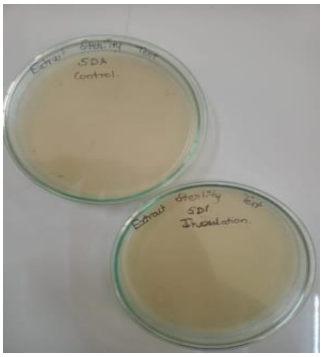

Figure No :27 (No growth observed in SDA Agar plates)

SUMMARY AND CONCLUSION

This project work investigated and concluded that the Formulated herbal extract could be an alternate source of the harmful chemical disinfectants. Rosemary contains Rosmarinic Acid, This is found in high concentrations in rosemary and exhibits potent antimicrobial activity against various bacteria, fungi, and viruses. It works by disrupting the cell membranes of microorganisms and interfering with their growth and replication. Thyme contains thymol, it disrupts microbial cell membranes, interferes with cellular respiration, and inhibits microbial enzyme activity, leading to the inhibition of microbial growth and proliferation. Oregano contains carvacrol, this works by disrupting microbial cell membranes, interfering with cellular metabolism, and inhibiting microbial enzyme activity. The herbal formulation works against the isolated pathogens efficiently when compared to the commercially brought Disinfectants. And also a Eco-friendly, Sustainable product.

5. REFERENCE

1. Tully TN Jr: Birds. In Manual of exotic pet practice. Edited by Elsevier. St. Louis, Missouri (USA): Elsevier; 2009:250–298.
2. Carlson JC, Engeman RM, Hyatt DR, Gilliland RL, DeLiberto TJ, Clark L, Bodenchuk MJ, Linz GM: Efficacy of European starling control to reduce Salmonella enterica contamination in a concentrated animal feeding operation in the Texas panhandle. BMC Vet Res 2011, 7:9.
3. Vanrompay D, Harkinezhad T, Van De Walle M, Beeckman D, Van Droogenbroeck C, Verminnen K, Leten R, Martel A, Cauwerts K: Chlamydophilapsittaci transmission
4. Boseret G, Carere C, Ball GF, Balthazart J: Social context affects testosterone- induced singing and the volume of song control nuclei in male canaries (Serinus canaria). J Neurobiol 2006, 66:1044–1060.
5. Belchior E, Barataud D, Ollivier R, Capek I, Laroucau K, De Barbeyrac B, Hubert B: Psittacosis outbreak after participation in a bird fair, Western France, December 2008. Epidemiol Infect 2011, 139:1637–1641.
6. Boseret G, Carere C, Ball GF, Balthazart J: Social context affects testosterone- induced singing and the volume of song control nuclei in male canaries (Serinus canaria). J Neurobiol 2006, 66:1044–1060.
7. Valiente Moro C, Thioulouse J, Chauve C, Normand P, Zenner L: Bacterial taxa associated with the hematophagous mite Dermanyssus gallinae detected by 16S rRNA PCR amplification and TTGE fingerprinting. Res Microbiol 2009, 160:63–70
8. Valiente Moro C, Chauve C, Zenner L: Vectorial role of some dermanyssoid mites (Acari,

Mesostigmata, Dermanyssoidea). Parasite 2005, 12:99–109.
9. Valiente Moro C, De Luna CJ, Tod A, Guy JH, Sparagano OAE, Zenner L: The poultry red mite (Dermanyssus gallinae): a potential vector of pathogenic agents. Exp Appl Acarol 2009, 48:93–104.
10. Blitvich BJ: Transmission dynamics and changing epidemiology of West Nile virus. Anim Health Res Rev 2008, 9:71–86.
11. Turell MJ, O'Guinn M, Oliver J: Potential for New York mosquitoes to transmit West Nile virus. Amer J Trop Med Hyg 2000, 62:413–414.
12. Nemeth N, Young G, Ndaluka C, Bielefeldt-Ohmann H, Komar N, Bowen R: Persistent West Nile virus infection in the house sparrow (Passer domesticus). Arch Virol 2009, 154:783–789.
13. Pfeffer M, Dobler G: Emergence of zoonotic arboviruses by animal trade and migration. Parasit Vectors 2010, 3:35.
14. Comstedt P, Bergström S, Olsen B, Garpmo U, Marjavaara L, Mejlon H, Barbour AG, Bunikis J: Migratory passerine birds as reservoirs of Lyme borreliosis in Europe. Emerg Infect Dis 2006, 12:1087–1095
15. Mathers A, Smith RP, Cahill B, Lubelczyk C, Elias SP, Lacombe E, Morris SR, Vary CP, Parent CE, Rand PW: Strain diversity of Borrelia burgdorferi in ticks dispersed in North America by migratory birds. J Vector Ecol 2011, 36:24–29
16. Van Droogenbroeck C, Beeckman DSA, Verminnen K, Marien M, Nauwynck H, Boesinghe LT, Vanrompay D: Simultaneous zoonotic transmission of Chlamydophila psittaci genotypes D, F and E/B to a veterinary scientist. Vet Microbiol 2009, 135:78–81
17. Perkins LEL, Swayne DE: Varied pathogenicity of a Hong Kong-origin H5N1 avian influenza virus in four passerine species and budgerigars. Vet Pathol 2003, 40:14–24.
18. Wu Y, Du PC, Li WG, Lu JX: Identification and molecular analysis of pathogenic yeasts in droppings of domestic pigeons in Beijing, China. Mycopathologia 2012, 174:203–214.
19. Brilhante RSN, Castelo-Branco DSCM, Soares GDP, Astete-Medrano DJ, Monteiro AJ, Cordeiro RA, Sidrim JJC, Rocha MFG: Characterization of the gastrointestinal yeast microbiota of cockatiels (Nymphicus hollandicus): a potential hazard to human health. J Med Microbiol 2010, 59:718–723.
20. Lugarini C, Goebel CS, Condas LAZ, Muro MD, De Farias MR, Ferreira FM, Vainstein MH: Cryptococcus neoformans isolated from Passerine and Psittacine bird excreta in the state of Paraná, Brazil. Mycopathologia 2008, 166:61–69.
21. Papini R, Girivetto M, Marangi M, Mancianti F, Giangaspero A: Endoparasite infections in pet and zoo birds in Italy. Sci World J 2012, 2012:25312.
22. Kogevinas M, Anto JM, Sunyer J, Tobias A, Kromhout H, Burney P (1999): Occupational asthma in Europe and other industrialized areas: a population based study. Lancet 353:1750–1754.
23. Omland O (2002): Exposure and respiratory health in farming in temperate zones–a review of the literature. Ann Agric Environ Med 9:119–136.
24. Radon K, Danuser B, Iversen M, Jo¨rres R, Monso E, Opravil U et al (2001): Respiratory symptoms in European animal farmers. Eur Respir J 17:747–754
25. Macan J, Kanceljak B, Plavec D, Milkovic´-Kraus S (2003): Differences in mite fauna between the continental and Mediterranean climates of Croatia: microscopy and DustscreenTM test findings. Allergy 58:780–783.
26. Davor Rimac; Jelena Macan; Veda M. Varnai; Marija Vučemilo; Kristina Matković; Ljerka Prester; Tatjana Orct; Ivančica Trošić; Ivan Pavičić (2010):Exposure to poultry dust and health effects in

poultry workers: impact of mould and mite allergens. , 83(1), 9–19.
27. Anni McLeod, Nancy Morgan, Adam Prakash, Jan Hinrichs: Economic and social impact of avian influenza.
28. Milan Brambhatt, World Bank, September 2005. “Avian Influenza: Social and Economic Impacts”.
29. World Bank (2004): “The impact of the avian influenza epidemic on theVietnamese economy: an estimate by the World Bank in Vietnam”.
30. Gorman, Sean, and Eileen Scott. "Chemical disinfectants, antiseptics andpreservatives." Hugo and Russell’s Pharmaceutical Microbiology (2004): 285-305.
31. McDonnell, Gerald, and A. Denver Russell. "Antiseptics and disinfectants:activity, action, and resistance." Clinical microbiology reviews 12.1 (1999): 147-179.
32. Forsberg K, Van den Borre A, Henry III N, Zeigler JP. Quick Selection GuidetoChemical Protective Clothing, 7th Edition. Wiley. 2020.
33. Padmaja, Indugula Jyothi. "Comparative evaluation of antimicrobial activity ofnewer herbal disinfectant formulations on microbial flora."
34. Fung, D.Y.C.; Taylor, S.; Kahan, J. Effect of butylated hydroxyanisole (BHA) and *buthylated hydroxytoluebe (BHT) on growth and aflatoxin production of Aspergillus flavus. J. Food Saf.* 1977, *1*, 39–51.
35. Xu, J., et al : "The antibacterial mechanism of carvacrol and thymol against Escherichia coli." Letters in applied microbiology 47.3 (2008): 174-179.
36. Cushnie, TP Tim, and Andrew J. Lamb. "Antimicrobial activity of flavonoids." International journal of antimicrobial agents 26.5 (2005): 343-356.
37. Lan, Weiqing, et al. "Antimicrobial activity and mechanism of oregano essential oil against Shewanella putrefaciens." Journal of Food Safety 42.1 (2022): e1295
38. Jansson, Linda, et al. "Impact of swab material on microbial surface sampling." Journal of Microbiological Methods 176 (2020): 106006.
39. Bangert RL, Cho BR, Widders PR, Stauber EH, Ward AC. A survey of aerobic bacteria and fungi in the feces of healthy psittacine birds. Avian Dis. 1988 Jan-Mar;32(1):46-52. PMID: 3382379.
40. Mendes JF, Albano AP, Coimbra MA, Ferreira GF, Gonçalves CL, Nascente Pda S, de Mello JR. Fungi isolated from the excreta of wild birds in screening centers in Pelotas, RS, Brazil. Rev Inst Med Trop Sao Paulo. 2014 Nov-Dec;56(6):525-8. doi: 10.1590/s0036-46652014000600012. PMID: 25351548; PMCID: PMC4296874.
41. Hirondart, M.; Rombaut, N.; Fabiano-Tixier, A.S.; Bily, A.; Chemat, F. Comparison between Pressurized Liquid Extraction and Conventional Soxhlet Extraction for Rosemary Antioxidants, Yield, Composition, and Environmental Footprint. *Foods* 2020, *9*, 584.
42. Kowalska-Krochmal B, Dudek-Wicher R. The Minimum Inhibitory Concentration of Antibiotics: Methods, Interpretation, Clinical Relevance. Pathogens. 2021 Feb 4;10(2):165. doi: 10.3390/pathogens10020165. PMID: 33557078; PMCID: PMC7913839.
43. England, Joseph W. "The phenol coefficient method of testing disinfectants." The Journal of the American Pharmaceutical Association (1912) 2.8 (1913): 955-95.