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Comparing the effectiveness of egg disinfectants against bacteria and mitotic indices of developing chick embryos

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Abstract Total bacterial counts on hatching eggshell surface were significantly ($P < 0.05$) reduced as a result of using all disinfectants with different concentrations and formaldehyde fumigation treatments compared with those for eggs before treatment except for those subjected to water only which are considered as control with water. Chemical disinfectants significantly reduced the eggshell total bacterial count from 7.07 Logs to 2.41 Logs with 65.9% reduction and decreased again to 1.96 Logs with 72.3% reduction before setting in the incubator. Also, natural disinfectants significantly reduced the total bacterial count from 7.0 Logs to 1.86 Logs with 73.7% reduction and decreased again to 1.34 Logs with 81% reduction before setting in the incubator. Whereas, treatment with formaldehyde fumigation significantly reduced the bacterial count from 7.07 Logs to 2.53 Log with 64.2% reduction, but the bacterial count had increased numerically again during storage and before setting in the incubator to 4.20 Logs. Chemical disinfectant effects on developing chick embryos resulted in retarded growth as reflected by malformed limbs and beaks and muscle weakness was seen in a few hatched chicks. The mitotic indices of the spinal cord for chicks from egg treated by cumin 0.2% at 3rd and 4th day of age are slightly higher being 5.5% and 4.8% respectively, than those for other treatment and control groups. The mitotic index revealed that there was a significant ($P < 0.05$) difference between all disinfection and control groups on days 4, 7 and 10 of incubation with respect to skin systems, whereas skin system of newly hatched chicks did not demonstrate any significant differences between mitotic indices of experimented groups.

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Mitotic indices of embryonic dermal system on days 4 and 10 of incubation were slightly higher for natural disinfectant (being 4.7 and 0.1) compared with those for the chemical disinfectant (being 4 and 0.6), formaldehyde fumigation (being 3 and 0.4) and control group (being 4 and 0.9).

Introduction

Disinfectants are an essential part of infection control practices and aid in the prevention of disease outbreaks on farms (Dvorak, 2005). Currently there are over 5000 antimicrobial products registered with the Environmental Protection Agency as "antimicrobial pesticides", which are substances or mixtures of substances used to destroy or suppress the growth of harmful. Many parent compounds have been made more effective, stable and less irritating by the addition of other chemical groups. Therefore, it is not appropriate to generalize the activity of a parent compound such as iodine or phenol, upon the commercial derivatives available (Ralph, 2003). There are many disinfectants to choose but they basically fall into a few categories based on the active ingredients and abilities to kill different micro-organisms. Alcohol compounds are fast acting and highly effective against both Gram positive and Gram negative bacteria but have no residual activity such as, ethyl alcohol (ethanol, alcohol), isopropyl alcohol (isopropanol, propan-2-01) and *n*-propanol (McDonnell and Russell, 1999; Dvorak, 2005; Ewart, 2001; Turpin, 2013). Formaldehyde (CH_2CO, formalin, formol) is commonly used as a disinfectant, as it is cheap, not corrosive, and kills most bacteria and fungi (including their spores) (Acklund et al., 1980; Williams, 1969; Russell, 1976; Cadirci, 2009). Chlorhexidine compounds can kill microorganisms by damaging outer cell layers (McDonnell et al., 1999; Quinn, 2001). Sodium chloride is reported for wide antibacterial activity and low toxicity toward man and animal (Grooms, 2003). Virkon-S was essentially ineffective against the inoculated microorganisms (Scott and Swetnam, 1993b). Hydrogen peroxide also, has been used as a satisfactory disinfectant for inanimate materials (Scott and Swetnam, 1993a; Sheldon and Brake, 1991; Mansour, 2001; Sullivan and Krieger, 1992). Researchers have been interested in biologically active compounds isolated from plant species for the elimination of pathogenic microorganisms because of the resistance that microorganisms have built against antibiotics (Nychas, 1995; Essawi and Srour, 2000; Singh et al., 2001). The essential oil of oregano has anti-bacterial (Baydar et al., 2004; Vagi et al., 2005), anti-oxidant (Gouladis et al., 2003; Tepe et al., 2004), anti-fungal (Muller et al., 1995; Bouchra et al., 2003), cytotoxic (Sivropoulou et al., 1996; Wilson et al., 1997), insecticidal (Traboulsi et al., 2002) and nematicidal properties (Oka et al., 2000), the cumin seed contains powerful compounds. These natural constituents possess a remarkable antioxidant, antitoxic, anti-microbial, anti fungal, anti-parasitic, anti-spasmodic and diuretic actions (Tepe et al., 2005). Sanitizers and disinfectants that are most critical to the normal development of the embryo are those that occur before and during incubation and hatching processes (Wilson, 1991; Meijerhof, 2000). The magnitude of the mitotic index is a reliable indication of the rate of cell proliferation. As a general rule, when the mitotic index is high, proliferation is rapid and when it is low, the birth rate of cells is also low. El-Zayat (1974) and Michael et al. (1991) reported that during organogenesis the mitotic index is high at the time of proliferation and drops sharply by the onset of cellular differentiation. Rizk (1994) also concluded that the cell division of the nervous system could be affected by the egg abnormalities and then the survival and development of the embryos and finally hatching power. The present study was carried out to investigate the effect of chemical and natural egg disinfections against bacteria, embryonic development, embryonic mitotic indices in Bandarah local strain.

Materials and methods

A total of 1442 hatched eggs from Bandarah chicken strain were used in this experiment. Hatched eggs were divided into two divisions: firstly, forty two hatched eggs for bacterial count, secondly, 1400 eggs for embryonic inspection. The eggs for each division were divided into 14 groups, which represent the disinfectants used (Tables 1a and 1b), as disinfectants from chemical sources and others from natural sources with their combinations, formaldehyde fumigation and control groups. Each group for studying the development of chick embryos contains 100 developing and hatching eggs.

Egg from this group was treated with formaldehyde fumigation (triple strength), approximately 1 g potassium permanganate ($KMnO_4$) to 2 ml formalin (CH_2CO) per 1 m^3. Triple strength formaldehyde gas was produced inside the setter for 20 min (USDA, 1985). For embryonic study, three incubated eggs from Bandarah chicken strain were selected randomly representing each trial of experiment. Each egg was weighed and opened on days 3, 4, 7, 10, 13 and 21, then the embryos were separated from the remaining egg contents. Three developing embryos at each day of the preceding days of incubation for each experimental and control groups were used for determination of morphological examination and mitotic index as kinetic parameter of the cell cycle in two regions of the nervous system and skin .The region of the nervous system was the spinal cord. For histological preparation and studying, embryos were rinsed in saline water and fixed in Bouin's fluid for 24 h as described in the method of Gabe (1976). Fixation consists of the following compositions: saturated aqueous solution of picric acid 100 parts, formaldehyde solution 25% parts and five parts of glacial acetic acid were added promptly before using. This solution acts as a fixative and/or preservative. After fixation, embryos were thoroughly washed with 70% ethyl alcohol. Then they were dehydrated through an ascending series of alcohol then cleared in xylene and embedded in paraffin. Paraffin blocks were treated, fixed over the block holder of the microtome and serially sectioned at 4 and 5 u. The obtained paraffin ribbons containing the serial sections were cut into pieces of 5 cm long and mounted over a slide placed over a hotplate adjusted at 40 °C. The mounted