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REVIEW ARTICLE

Antimicrobial Applications in Agriculture: A Review

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Abstract

Antimicrobials receive a great response in agriculture industries. Rapid growth of agriculture products has encouraged huge development of antimicrobial agent to improve growth and health of animals and plants. This paper focuses on the applications of antimicrobial agents such as antibiotics which have been widely used in plants and crops, poultry, aquamarine, and livestock production to inhibit microbial infection by various types of pathogenic microorganisms such as bacteria, viruses, fungi, and parasites. Research related to disease treatment and prevention in agriculture product through proper use of antimicrobial agents is very crucial due to the emergence of bacterial resistance towards antimicrobial drugs. Global concern on the spread of bacterial resistance has gained interest on the research for high potential of antimicrobial agent such as essential oil as it is claimed to have strong antimicrobial activity and safe for humans, animals and plants.

Keywords: Antimicrobials, Antimicrobial agents, Essential oil.

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INTRODUCTION

Antimicrobial agent has been used for more than 100 years[1]. In some parts of the world, antimicrobial agent is far greater applied in animals and plants than in human[2]. It is estimated that almost 29.5 million pounds of antimicrobials are used in animals while 40 thousand pounds of antimicrobials are applied in plant agriculture every year[3,4]. Continuous use of antimicrobial agents in agriculture sector will occur with the rise of economic growth as they offer considerable benefits, in terms of animal and plant welfare as well as food production for human consumption. The purposes of antimicrobial agents in agricultures are mainly to improve health, to stimulate quicker growth of animals and plants as well as to preserve food[5].

Recently, antimicrobials have huge applications in agriculture which include the applications in plants and crops, aquamarine (shrimp and fish), livestock (cattle and pigs) and poultry production (chicken) [6,7,8,9]. It is essential to involve antimicrobial agent in agriculture products as humans and animals are prone to be affected by different or even same pathogens[10]. Several types of antimicrobial agents that have been used in agricultures include antibiotics, disinfectant (antimicrobial agent used only on inanimate objects), antiseptics, bactericidal (agent that kills bacteria), bacteriostatic (agent that inhibits the growth of bacteria), chemotherapeutic, as well as essential oils[1,11,12,13]. Among these antimicrobial agents, antibiotics are the most widely used in agriculture because it is cheaper and can be easily obtained. However, frequent use of antibiotics in animals and plants had eventually led to the occurrence of bacterial resistance which had triggered the spread of bacterial outbreaks hence risking animals and plants production[14]. The use of antibiotics in animals and plants has led to a serious health concern among consumers as it cannot provide safe animals or plants production for human consumption. Thus, it is crucial to know the limitations of each antimicrobial agent used in animals and plants in order to secure the safety of agriculture products.

Through the advancement of current technology and economic growth, there is high demand for effective, cheaper, and safe antimicrobial agent to promote healthy well growth of animals and plants in agricultures. This is because agriculture based products are big contributors for the spread of infectious diseases that can affect human health. Thus, it is important to study and have deep knowledge on the proper use of antimicrobial agents in agriculture to avoid the spread of drug-resistant bacteria among animals, plants, and humans.

Antimicrobial Application in Plant / Crops

In plants and crops, diseases caused by bacteria are less prevalent than virus and fungal diseases. In the United States, antimicrobials were applied on 53,000 hectares every year by spray treatment[15]. However, the uses of antimicrobials such as antibiotics in prophylactic treatment are limited in type and availability due to its poor efficacy and environmental concerns. Occurrence of antimicrobial resistance in plants and crops has begun to arise since early of 1960s[16]. It worsened when monitoring of bacterial resistance towards antimicrobial drugs are occasionally done. However, there is no human health effects have been documented since inception use of antimicrobials in plants in the last six decades[17].

Citrus, for example, has high water content and nutrients, making it very susceptible to infection by microbial pathogens like *Penicillium digitatum* (*P. digitatum)* during the period between harvest and consumption[18]. *Bacillus subtilis* strains and their metabolites are claimed to have strong antimicrobial activities towards green mold pathogen (*P. digitatum*) of the citrus fruit[19]. There are twenty strains of *Bacillus* species isolated from soil showing antagonistic activities *in vitro* towards the *P. digitatum* pathogen. Volatile compounds produced by *B. subtilis* strains can exhibit 30-70% inhibition of fungal growth. In addition, an ethanol extract from a *B. subtilis* 155 produced the best inhibitory effect on mycelial growth and spore germination of the fungus compared to *B. subtilis* 121, 162, 153 and 176, *B. megaterium* 202, *B. anthracis* 146 and *B. pumilus* 147 and 122[20]. Thus, *B. subtilis* 155 is considered to be potent biological control agents to suppress the growth of *P. digitatum* in postharvest protection of citrus. Postharvest application of microbial antagonists by spraying, dipping or drenching is the most used and practical method for controlling postharvest diseases of citrus fruit[18].

A common soil borne fungus called

Ganoderma is able to infect oil palm trees, which is increasingly becoming a concern for plantation operators in Malaysia. *Ganoderma* is a genus of wood-decaying fungi found throughout the world, affecting all types of woody trees, including oil palms[21]. Figure 1 shows example of *Ganoderma* Butt Rot (Basal Stem Rot (BSR)) disease. It was reported that the spread of BSR disease can kill 80% of oil palm stands in the infected area within 15 years of the crop being established[22]. The fungus will gradually colonize the trunk and rot it from inside, making the disease lethal. *Ganoderma* is able to cause significant yield losses well before it has actually killed an oil palm, while its spores can spread through wind and water to ever increasing areas of a plantation once it has been introduced. It can arrive on the soil/roots of a transplanted seedling originating from a nursery, or be endemic to a particular location where a plantation was developed[24]. In contrast, *Trichoderma* is a non-pathogenic fungi found in soil which is used as a fungicide to control *Ganoderma* successfully. As a biological control agent, it is environmental friendly. *Trichoderma* has unique mechanisms to control pathogens. The mechanisms include competition for nutrient and space, induced resistance of the host plant, tolerance to stress and solubilization of inorganic nutrients. Palm press fiber was used as the carrier (food substrate) of *Trichoderma*[21,23]. Out of seven fungicides, bromoconazole and hexaconazole were effective in delaying the death of infected palms compared to benomy & thiram, triadimefon, triadimenol and tridemorph. To improve the delivery of chemical to the target sites for controlling BSR, a pressure injection apparatus was used[22].

Fig. 1. Basal Stem Rot disease of the oil palm tree (Elliot *et al.*, 2004)

Similarly, antibiotics such as streptomycin, oxytetracycline, oxolinic acid and gentamicin are essential to control bacterial diseases of plants like fire blight of pear and apple caused by *Erwinia amylovora*. Springtime antibiotic sprays suppress the pathogen growth on flowers and leaf surfaces before the infection occurs. It is applied when the risk of diseases and infections is high. Antibiotics are active on plants for less than a week and no longer effective after the infection. In the United States, antibiotics have been totally necessary for crop protection for more than 50 years without any reports of adverse effects on human health or continuous impacts on environment[26].

Antimicrobial Application in Aquamarine

A probiotic bacterium, *B. cereus* isolated from the gut of wild shrimp called *Penaeus monodon* (*P. monodon*) rendered maximum hostile activity against shrimp pathogens and was capable of producing extracellular enzymes. A study by NavinChandran and co-workers found that lyophilized *B. cereus* was efficient in stimulating the growth and immunity of shrimp. It is also showed that the shrimp having fed of higher concentration of lyophilized probiotic strain produced higher total haemocyte count, phenoloxidase activity, respiratory burst activity, lysosome activity, plasma protein concentration and bactericidal activity compared to other diets with lower concentrations[27].

Another common aquamarine disease is bacterial gill disease by *Flavobacterium branchiophilum* bacteria which infect fish such as salmon[28,29,30]. Normally, it occurs in young cultured salmonids and fish cultured under conditions of high organic loading[31]. It can also occasionally be found in aquarium fish. The disease can be initiated by crowding and poor water quality that particularly has high organic loads, high ammonia levels, and silt[32,33,34]. Once infected, gills appear swollen and mottled, with patchy areas of bacterial growth. Bacterial gill disease is commonly treated with chloramine T and benzalkonium chloride. In addition, adequate oxygen supply will be a helpful supportive therapy[35]. Broad-spectrum antibacterials are effective to control the outbreak of other fish diseases such as Enteric

Redmouth Disease which is caused by *Yersinia ruckeri,* Furnculosis and Vibriosis. For Coldwater Diseases and Rainbow Trout Fry Syndrome (RTFS), broad-spectrum antibacterials have been partially ineffective in controlling the outbreak, but the improvement of the environment and multiplying recommended doses of antibacterial have shown benefits[34].The application by water-borne route is necessary if the fish refuse to eat as it would be unlikely to consume any medicated food. This method has exposed the fish to suspensions of antibiotics for the prescribed period.

Antimicrobial Application in Poultry

In poultry, antimicrobial agent is used in prophylactic treatment as an agent to prevent the disease from occurring[36]. In early 1940s, antimicrobials were used in animal poultry at low concentrations as feed additives for growth promotions. It was discovered that chickens showed a faster growth rate when they are fed with antibiotics like tylosin, chlorotetracycline, tetracycline, bacitracin, oxytetracyclin and penicillin[37]. Besides, an antibiotic such as tetracycline is very excellent against Gram positive and Gram negative bacteria[38]. It was reported that chronic respiratory disease caused by *Mycoplasma gallisepticum* and *M. synoyiae* bacteria have been treated with several antibiotics like macrolides, lincomycin, tetracyclines, spectinomycin, and timulin[39,40,41]. During the past 50 years, antibiotics were widely used in poultry production as therapeutic agents to treat bacterial infections as the infections can reduce the performance of chickens and cause diseases[42]. Extensive use of antibiotics should be regulated by specific veterinarian or pharmaceutical legislation in order to prevent the spread of antimicrobial resistance as well as bacterial infections.

Other than antibiotics, veterinary drugs such as coccidiostats are introduced in the feed to promote intensive production in commercial poultry for over 60 years. In early 1980s, a new group of coccidiostat which is known as ionophores was used to treat coccidiosis in chickens. Basically, ionophores can kill the parasite by interfering with the passage of ions across the cell membrane, whereas chemicals act by inhibiting different biochemical pathways of the parasite[43]. However, ionophores have limited antibacterial activity against *Clostridium* bacteria species. For this reason, ionophores were used exclusively as coccidiostats. Similarly, sulfonamides are also used in chickens for the treatment and prevention of coccidiosis[44,45]. The significant use of ionophores as antimicrobial agent has increased in the European Union (EU) since antimicrobial growth promoters were banned[46]. It is still mainly used worldwide as these agents are not used as human medicine.

There are a few methods that have been used in preventing or treating the diseases in animal poultry through animal feeding, drinking and injection. Besides through animal feeding, antibiotic treatment can be applied either by *in ovo* injection or by dipping eggs produced by genetic stocks. For *in ovo* injection, the antibiotic is injected either through amniotic cavity or directly into the chicken's embryo within the air chamber, while egg dipping is conducted by dipping the eggs in antibiotic solution through depression produced by vacuum pump[47]. Apart from antibiotics, another way to treat hazardous microbes in animal poultry is by using natural products such as fungal extracts, probiotics, antioxidants, essential oils, herbal extracts and medical plants[43,48,49,50]. Various fruit and herb plants including plum, cranberries, pomegranate, bearberry, grape seed extract, pine bark extract, rosemary, oregano, green tea, and other species may function as antimicrobial and antioxidants in meat and poultry products[51,52]. These natural products provide safer and cheaper alternative to antibiotics.

Antimicrobial Application in Livestock

Antimicrobial agents possess major contributions towards health and welfare of livestock animals such as dairy cattle, pigs, sheep and goats. Antimicrobial agents are also used in livestock production to maintain health and productivity of the animals, contributing to food security, nutrition and livelihoods for farmers. Population growth, urbanization and rising incomes of low developed countries drive up the demand for animal-source food[53]. Farmers are intensifying their production that often leads to the increasing use of antimicrobial agents such as antibiotics. When administered sub-therapeutically in low dosages, antimicrobial agents such as antibiotics may promote a prophylactic effect and better animal growth.

Flavophospholipol is the most

common antibiotic growth promoter used with livestock in different continents over the world. In the USA, pigs are exposed to bacitracin, pleuromutilins, quinoxalines, flavophospholipol and virginiamycin as growth promoters. For cattle, flavophospholipol, virginiamycin and monensin are used. In Australia, the growth promoters used for pigs are flavophospholipol, kitasamycin, tylosin, olaquindox and virginiamycin. Lasalocid, monensin, narasin, flavophospholipol, oleandomycin and salinomycin are used for cattle. Meanwhile, in the EU, flavophospholipol, monensin and salinomycin are used for cattle and pigs. Other than that, avilamycin is also used for pigs[54]. This situation had led to the interest of using antibiotics in livestock production.

Animals that are suffering from respiratory infections such as Anthrax disease are difficult to treat and typically have high chances of death. Ruminants such as cattle, sheep and goats are the most susceptible group to Anthrax infection. This is followed by pseudoruminants, carnivores and scavengers in the order of lower susceptibility. Anthrax disease, which is caused by large spore-forming rectangular shaped bacterium, *B. anthracisar* is commonly treated using antibiotics like ciprofloxacin, tetracycline, erythromycin and penicillin[56]. However, the response to the treatment by using antibiotics may vary from time to time which only obtain the best result when the drugs are administered early during an outbreak[57].

Another common disease involved in livestock production is mastitis. It is caused by various species of *streptococci*, *staphylococci*, and gram-negative rods bacteria, especially lactose-fermenting organisms of enteric origin that is commonly called coliforms[58]. It can be treated using beta lactams, tetracyclines, lincomycin and combination of trimethoprim-sulfonamide[59]. Clinical mastitis is an inflammatory response to the infection causing visibly abnormal milk through its color and fibrin clots. Commonly, the affected animal will be given an antibiotic by infusing directly into the infected gland. In this case, the removal of the primary growth medium of the bacteria such as the milk may enhance rate of recovery from infection[60].

The use of antimicrobials to treat bacterial diseases among animals will disqualify the organic status. This condition limits the treatment choices for animals with organic management. However, vaccines and anti-inflammatory drugs such as aspirin and flunixin are permitted by the US organic standards. In order to preserve the organic status, no antimicrobials shall be used to treat the animals. Clinical mastitis is conventionally treated by intramammary infusion of antimicrobial compounds such as cephapirin, pirlimycin and amoxilin. In contrast, this treatment is not a practice for organic dairy herds. Instead, the farmers opt for alternative treatment, for example, homeopathy[55].

Besides that, the therapeutic treatment of some major diseases in pigs also requires antibiotics. For example, infection of enterotoxigenic *Escherichia coli* in piglets and weaner pigs are typically treated by using amoxicillin, spectinomycin, apramycin and neomycin through feed medication. Trimethoprim is also used as an injectable antibiotic[61].

Essential Oil as Antimicrobial Agent in Agriculture

Currently, essential oil offers a great alternative to the use of antimicrobial drugs since overuse of antibiotics had led to the development of antimicrobial drug-resistant bacteria. Essential oil possesses excellent antimicrobial properties against fungi and bacteria with the presence of several antimicrobial components including phenolics and bioactive volatile compounds[62,63,64]. These chemical components have great potential to treat infectious disease and can act as food preservative agent[65,66]. They are typically present in the source tissue from twigs, leaves, barks, or wood pulp of higher plants. Essential oil was also reported to be found in bryophytes like liverworts[67]. Essential oils have been used in some industries since decades due to high availability, safety, and strong efficacy[62,68]. Thus, intense research and discovery on essential oil as new and effective antimicrobial agents can be developed to be used in wide industries such as food and agriculture.

In plants and crops, essential oils are commonly applied as an antifungal agent to inhibit the growth of fungi among plants species. Essential oil from red thymes, cinnamon leaves, and clove buds demonstrate highest antifungal activities towards *Botrytis cinerea* fungi[14]. In another study, Singh A.K. and co-workers claimed that essential oil from *Cymbopogon martinii*, *C. olivieri*, and *Trachyspermum ammi* can effectively inhibit fungi species including *Helminthosporium oryzae*[69]. Moreover, essential oil also possesses strong antiviral activity towards plant viruses. It is significantly proven when essential oil from cajuput (*Melaleuca leucadendron* (L.) L.), myrtus (*Myrtus communis* L.) and winter savory (*Satureja montana* L.) were tested against *Tobacco mosaic virus* by inoculation in plant host system[70]. Other than preventing the spread of viruses, essential oil also has great potential in reducing the herbivorous insect like spiders, caterpillars, whiteflies, and aphids which can damage crops and disrupt the plant growth[71,72,73,74]. It was successfully applied in inhibiting the grain pests and biting flies through its natural aroma[75]. Thus, essential oil provides good alternative in plants and crops agriculture as it can provide many benefits in protecting and promising well growth of the plants.

Essential oil has also gained interest in poultry industry. Essential oil provides effective solution to the spread of bacterial infections caused by *E. coli*, *Salmonella*, and *Campylobacter* genus which infect poultry product such as chicken eggs[76]. It was reported that essential oils can demonstrate strong antibacterial activities towards Gram-positive bacteria as well as Gram-negative bacteria[77,78,79]. Besides, essential oil can be used as a growth promoter to increase the performance of broilers in terms of weight, amount of feed intake, egg production, and rate of growth. In addition, essential oils of garlic, onion and mint have antiparasitic activity which isnoticed to be effective against intestinal parasite in an animal's body. Essential oil possesses high antioxidant and anti-inflammatory activities due to the presence of phenolic compounds[80,81]. This provides natural antioxidant on chicken meat. Essential oil can also promote better oxidative status by reducing peroxidation of lipid in the chicken's muscles. This is because susceptible thigh muscles will have potential to oxidize due to the high content of polyunsaturated fatty acids which will generate peroxides, lipids, oxysterols, as well as malondialdehyde[82].

In livestock production, essential oil is used as animal dietary supplement due to their decreasing intake of novel food. It allows sheep to consume enough nutrient bases in their meal and enhance positive post-ingestive feedback in the animal's body[83]. Essential oil is applied as an antibacterial agent in dairy cows to inhibit the growth of Gram-positive bacteria that will be able to reduce biohydrogenation of fatty acid and produce higher conjugated linoleic acid trans10, cis12 with minimum effect of fatty acid in cow's milk[84]. Furthermore, it has potential to reduce the growth of protozoan in animal's body. According to the study by B. Lin et al., it was proven that sheep treated with essential oil combination of clove, oregano, cinnamon and lemon have lower protozoan population which is reduced more than 50% compared to untreated sheep[85]. Thus, high performance of essential oil towards animals may enhance good quality of livestock production.

Alternative of using essential oil is friendly to the environment, producers and consumers. It can lower the risk of developing resistance, such as what have been observed in chemical drugs like antibiotics[81]. Moreover, essential oils are safer and the raw materials are readily abundant in nature compared to commercial antimicrobial drug. Hence, addition of essential oils can serve as green antimicrobial agent for conservation and preservation of animals and plants in agriculture industries.

CONCLUSION

Regardless how much benefits of using antibiotics in agriculture, the risk of antimicrobial resistance from some antimicrobial drugs on animals, plants, and humans is still becoming a major concern worldwide. The use of antimicrobial agents such as antibiotic should be carefully inspected and studied before applying on animals and plants. Thus, alternative techniques by using safe and effective antimicrobial agents must be developed and investigated in agriculture industries using natural extract such as essential oil and protein as potential candidates. They offer potent antimicrobial agents in agriculture industries by showing strong antimicrobial activities against various microorganisms including bacteria, fungus, and parasite.

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