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COMPONENTS, MECHANISMS OF ACTION, SUCCESS UNDER DIFFERENT CONDITION AND MARKET AVAILABILITY OF BIOCONTROL AGENTS

¹Tariku Abena* & ¹Daniel Yimer

*Ethiopian Institute of Agricultural Research (EIAR), National Agricultural Biotechnology
Research Center (NABRC), Microbial Biotechnology Research Program, Holeta,
Addis Ababa, ETHIOPIA*

Abstract

Traditional Agricultural practices are increasingly being affected by diseases, pests, droughts, decreased soil fertility due to use of hazardous chemical pesticides, pollution and global warming. Thus this review focus components, success and market demands of biocontrol. There is a wide use of microbial pesticides for soil-borne plant pathogens causing serious diseases of crops. This review address the concern of resistance development of human and fruit pathogens is increased using antibiotics in food products. Biocontrols are usually formulated in the form of dry for direct application. There is a success using biological control agent against a wide range of soil borne pathogens under greenhouse and field conditions.

Keywords: Biological control agent, BT toxin, Post-harvest, Soil-borne plant pathogens.

Corresponding Author:

Tariku Abena

*Ethiopian Institute of Agricultural Research (EIAR),
National Agricultural Biotechnology Research Center (NABRC),
Microbial Biotechnology Research Program, Holeta,
Addis Ababa, ETHIOPIA*

E-mail: tarikuabena8@gmail.com

Phone: +251909311778



INTRODUCTION

Worldwide traditional agricultural practices are increasingly being affected by various problems such as diseases, pests, droughts, decreased soil fertility due to use of hazardous chemical pesticides, pollution and global warming. There is thus a need for some eco-friendly biocontrol agents that may help to resolve some of these problems. Biological control, the use of specific microorganisms that interfere with plant pathogens and pests, is a nature-friendly, ecological approach to overcome the problems caused by standard chemical methods of plant protection (Harman *et al.* 2004). At present, it becomes difficult to control soil born diseases by using single method (Hausbek and Lamour, 2004). There are several negative effects like; development of pathogen resistance, hazards to humans, damage to beneficial organisms and environmental pollution as a consequence of Using of chemicals to control soil borne pathogens. For sustainable production, pathogens still need to be controlled in order to ensure healthy plant establishment and growth (Gerhardson, 2002).

Therefore, in order to provide an alternative to chemical control developments of various biological control agents' methods are urgently needed. Among different biological approaches, use of the microbial antagonists like yeasts, fungi and bacteria could be promised, effectively, safely and eco-friendly in controlling many of soil borne pathogens (Gravel *et al.*, 2004). Many biological control agents such as *Trichoderma* spp. and *Bacillus* spp. could be effectively used in suppressing diseases caused by *Fusarium* spp. *Drechslerahalodes* and *Rhizoctoniasolanias* reported by many workers (Hashem and Hamada, 2002; Nourozian *et al.*, 2006; Abdel- Monaim, 2010). Modes of action for beneficial micro-organisms include direct parasitism of plant pathogens, competition for space or nutrients, or production of antibiotics, enzymes or plant hormones (Lugtenberget *al.*, 2003). This led to promote plant growth during the growing season as reported by Mercier and Manker (2005). However, up to date, only a few antagonist microorganisms have been identified as potential, effective bio-control agents against soil borne pathogens (Spadaro and Gullino, 2005). Also, these bioagents increased significantly due to seed germination and increased of plant growth in wheat and other many crops (Riunguet *al.*, 2007; Zafariet *al.*, 2008; El- Mohamedy *et al.*, 2011).

Therefore this paper presents the components, mechanism of action, successes under greenhouse and field condition and market availability of biocontrol agents.

Components

Bacteria

The members of the genus *Bacillus* are often considered as microbial factories for the production of biologically active molecules, some of which are potentially inhibitory for fungal growth (Schallmey *et al.* 2004). The most widely used microbial agents are subspecies and strains of *B. thuringiensis* (Bt), accounting for approximately 90 % of the biocontrols market in the USA (Chattopadhyay *et al.* 2004). Several commercial products based on various *Bacillus* species such as *B. amyloliquefaciens*, *B. licheniformis*, *B. pumilus*, and *B. subtilis* have been marketed as biofungicides (Fravel 2005). Pseudomonads are also extensively used as a biocontrol of pathogens in agriculture (Ganeshan and Kumar 2006). Several species of *Pseudomonas* are being used as biocontrol are;- *P. fluorescence*, *P. aeruginosa*, *P. syringae*, etc. Certain strains of *Pseudomonas aureofaciens* are being used against a range of plant pathogens including damping off and soft rots (Kloepper *et al.* 2004; Berg 2009).

Fungal

The well-known fungal biocontrol agents are *Trichoderma* that are acclaimed as effective, eco-friendly, and cheap. These biocontrol agents are identified to act against a large number of important soil-borne plant pathogens causing serious diseases of crops (Bailey and Gilligan; 2004). Fungal biocontrols used against plant pathogens include *T. harzianum*, which is an antagonist of *Rhizoctonia*, *Pythium*, *Fusarium*, and other soil-borne pathogens (Harman 2005). The *Trichoderma viride* has proved to be very promising against soil-borne plant parasitic fungi (Khandelwal *et al.* 2012). There are also naturally occurring entomopathogenic fungi such as *B. bassiana* Vuillemin and *Metarhiziumanisopliae* (Metchnikoff) that infect sucking pests including *Nezaraviridula* (L) (green vegetable bug) and *Creontiades* sp. (green and brown mirids) (Sosa-Goméz and Moscardi 1998).

Viruses

Viruses are host specific, infecting only one or a few closely related species, thus offering minimal off-target impacts (Raymond *et al.* 2005; Hewson *et al.* 2011). A bacteriophage is a virus that infects bacterial cell walls. If the virus attacks bacteria that cause plant disease, it can be used as a pesticide. Baculoviruses are particularly attractive for use as biocontrol due to their high host specificity. Each virus only attacks particular species of insects, and they have been shown to have no negative impacts on plants, mammals, birds, fish, or nontarget insects (D'Amico 2007).

Mechanism of action

According to Blakeman and Parbery 1977 postulate there are four basic mechanism of antagonisms. These include direct parasitism, the production of extracellular antibiotics or other substances, competition and stimulation of host defenses.

Parasitism

Parasitism or predation is process in which antagonist feeds on or within the pathogen, resulting in a direct destruction or lysis of propagules and structure (Bull *et al.*, 1998). As reported by Bonaterra *et al.*,(2003) the direct parasitism by the antagonist on the pathogen propagules has a great advantage in biological control systems, specifically in soil-borne and to a lesser extent foliar diseases. Methods to demonstrate parasitism include burying and retrieving propagules of the pathogen to isolate the antagonist (Gardener and Fravel, 2002). Mycoparasites dissolve their fungal hosts' cell walls and penetrate the cells by using fungal cell-wall-degrading enzymes like chitinases, glucanases and b-1,3-glucanase (Eladet *et al.*, 1983). *Candida saitoana* yeast cells, that associates with fungal hyphae caused cytological damage and degeneration of the cytoplasm of *B.cinerea mycelium* (El-Ghaouhet *et al.* 1998). The extracellular enzyme such as exochitinase and b-1,3-glucanase that produced by *Aureobasidium pullulans* from apple wounds could play a role in the biocontrol activity (Castoria *et al.*, 2001).

The virus that infects *Cryphonectria parasitica*, a fungus causing chestnut blight, which causes, a reduction in disease-producing capacity of the pathogen (hypovirulence) and this mechanism used as biocontrol for chestnut blight in many places (Milgroom and Cortesi 2004). There are several fungal parasites of plant pathogens, including those that attack sclerotia (e.g. *Coniothyrium minitans*) while others attack living hyphae (e.g. *Pythium oligandrum*). And, a single fungal pathogen can be attacked by multiple hyperparasites. For example, *Acremonium alternatum*, *Acrodontium crateriforme*, *Ampelomyces quisqualis*, *Cladosporium oxysporum*, and *Gliocladium virens* are just a few of the fungi that have the capacity to parasitize powdery mildew pathogens (Kiss 2003).

Competition

Competition is a situation in which two or more microbial populations are simultaneously demanded for the same resource like, nutrient and spaces. (Droby and Chalutz, 1994). Competition for these resources proposed as a potential mechanism of action in biological control systems (Spadaro *et al.*, 2010). The antagonist should fulfill requirement such as, presenting in large quantities at correct time and location and utilizing resources more efficiently than the

pathogen (Larkin *et al.*, 1998). In terms of competition for space, certain microorganisms (yeasts and bacteria) have the added advantage of the formation of an extracellular polysaccharide capsule that can promote adhesion to the fruit surface (Spadaro and Gullino, 2003). There are several studies that shows competition is a mechanism of biocontrol that is likely to be used by many antagonists like, yeasts and bacteria (Wisniewski *et al.*, 1989; Lindow and Brandl, 2003; Vero *et al.*, 2009; Spadaro *et al.*, 2010).

Antibiosis

Antibiosis is a process in which microorganisms are inhibited or destroyed by substances such as specific or nonspecific metabolites, lytic agents, or enzymes that are produced by another microorganism (Melinet *et al.*, 2007). To be effective, antibiotics must be produced in situ in sufficient quantities at the precise time of interaction with the pathogen (El-Ghaouth *et al.*, 2002). It was discovered that bacteriocins, which are antibacterial proteins, produced by bacteria, kill or inhibit the growth of other bacteria (Cleveland *et al.*, 2001). Bacteriocins function by forming pores in the membrane of target cells and depleting the trans-membrane potential. This results in the leakage of cellular materials (Cleveland *et al.*, 2000). One well-known example is Pyrrolnitrin, compound produced by some *Pseudomonas* spp that provided the chemical model for development of Fludioxonil, a broad spectrum fungicide used as seed treatment (Gardener and Fravel, 2002). The production of antimicrobials substances by bacterial strains (*Bacillus cereus*, *Bacillus licheniformis* and *Bacillus subtilis*) against several avocado post-harvest pathogens was reported by (Korsten, 2006). *Bacillus thuringiensis* is another well-known bacteria that produces toxic compounds; BT toxin (Gerhardson, 2002). Using these antibiotics in food products raises concerns like, resistance development of human and fruit pathogens these compounds (Melinet *et al.*, 2007).

Volatile metabolites Production

Volatile organic compounds are chemicals with low molecular weight, high vapour pressure, and low water solubility which allow them to easily evaporate into the air or 'off-gas'. Volatile compounds from the biological control agents can be an important factor of the inhibitory mechanism, especially under closed storage condition, such as ethylene, released by the metabolic activities of the antagonist. Effects will be recorded as changes in radial growth, spore formation and colony forming units of the target fungi, which include *P. expansum*, *B. cinerea* and *Rhizopus stolonifer* (Mercier and Jimenez, 2004). The potential of the volatile-producing fungus *Muscodor albus* for controlling post-harvest diseases of fresh fruit (apples and peaches) by

biological fumigation was investigated. In vitro tests showed that *M. albus* volatiles inhibited and killed a wide range of storage pathogens belonging to species of *Botrytis*, *Colletotrichum*, *Geotrichum*, *Monilinia*, *Penicillium* and *Rhizopus* (Mercier *et al.*, 2007). Because *M. albus* has a sterile mycelium and does not require direct contact with the crops to be treated, it could be an attractive biological fumigant for controlling post-harvest diseases. The volatile profile of *M. albus* colonized grain was measured by gas chromatograph connected to a flame ionization detector (GC-FID) and showed that 2-methyl-1-guatanol and isobutyric acids were the major volatile compounds found (Mercier and Jimenez, 2004). The fact that volatiles from *M. albus* kill most storage pathogens exposed in vitro opens up new possibilities to develop biofumigation as a post-harvest treatment for a range of commodities (Mercier *et al.*, 2007).

Market availability

The most widely used microbial pesticides are subspecies and strains of *B. thuringiensis* (Bt), accounting for approximately 90 % of the biopesticide market in the USA (Chattopadhyay *et al.* 2004). In India, *P. fluorescens* biopesticide is effectively being used against late blight of potato; it is available commercially under diverse brand names such as Krishi bio rahat, Krishi bio nidan, Mona, etc. Virulent cells of bacterial antagonist *P. fluorescens* are taken to prepare a biopesticide formulation that is effective against phytopathogen *Ralstonia solanacearum* (Bora and Deka 2007; Chakravarty and Kalita 2011). Apart from this *B. pumilus* QST 2808, *B. subtilis* QST GB03 are used for designing biopesticides, namely, Ballad® Plus and Kodiak®, for commercial purposes in the USA (Stewart *et al.* 2011). Several different commercially available biopesticides in the USA that are developed from *Pseudomonas* and effective against fungal phytopathogens are Spot-Less, At-Eze, Bio-Save 10LP, and Bio-Save 11LP (Nakkeeran *et al.* 2005; Khalil *et al.* 2013). The market share of baculoviruses is 6 % of all microbial pesticides (Quinlan and Gill 2006; Marrone 2007), and millions of hectares have been treated with registered baculovirus products over the years (Szewczyk *et al.* 2009; Kabaluk *et al.* 2010; Moscardi *et al.* 2011).

Successes under greenhouse and field condition

Gliocladium species are common soil saprobes and several species have been reported to be parasites of many plant pathogens (Viterbo *et al.*, 2007), for example, *Gliocladium catenulatum* parasitizes *Sporidesmium sclerotiorum* and *Fusarium* spp. *Gliocladium virens* has been used as a biological control agent against a wide range of soil borne pathogens such as, *Pythium* and *Rhizoctonia* under greenhouse and field conditions (Hebbar and Lumsden, 1999; Viterbo *et al.*,

2007). *Pythium oligandrum* has shown ability to control soil-borne pathogens both in the laboratory and in the field. *Pythium oligandrum* oospores have been applied as seed treatments which reduce damping-off disease caused by *P. ultimum* in sugar beet (Lewis et al., 1989; Khetan, 2001). The fungal biocontrol Arbuscular Mycorrhiza Fungi (AMF) have enhanced the resistance of cotton variety (Lumian 1) to *Verticillium* wilt under field conditions (QiagnZhag et al; 2018).

Formulation and development

Biocontrols are usually formulated as: dry formulations for direct application – dusts (DP), seed granules (GR), micro granules (MG), water dispersible granules (WG), and wet table powders (WP); emulsions, suspension concentrates (SC), capsule suspensions (CS); ultra low volume formulations (Knowles, 2006)

Dusts (DP)

DP are formulated by taking in an active ingredient on finely ground, solid mineral powder (talc, clay, etc.). This is an old formulation type that had been used for many years before granules were developed and they became restricted on the account of their adverse health impact on users. Other dusts are manufactured very simply and they are still used today in many parts of the world (Knowles, 2001)

Granules (GR)

GR are similar to dust formulations, except that granular particles are larger and heavier. Granular biocontrols are mostly used to apply products to soil in order to control weeds, nematodes, and insects living in soil, or for plant uptake by root. Once applied, granules release their active ingredient slowly. Some granules require soil moisture to release their active ingredient (Knowles, 2005; Lyn, 2010).

Wet table powders (WP)

WP are dry, finely ground formulations to be applied after suspension in water. Because of their dustiness during application, wet table powders are gradually suppressed by suspension concentrates or water dispersible granules, which have been the most widely used biocontrol formulations (Knowles, 2005).

Water dispersible granules (WG)

WG have been developed to overcome problems of dustiness of powder formulations. Water dispersible granules are designed to be suspended in water. Water dispersible granules are usually more expensive than older types of formulations (dusts, wettable powders) but their safety and

greater convenience regarding application make them still desirable for many users (Knowles, 2008).

Capsule suspension (CS)

CS are stable suspensions of micro-encapsulated active ingredient in an aqueous continuous phase, intended for dilution with water before use. Bio-agent as its active ingredient is encapsulated in capsules (coating) made of gelatin, starch, cellulose and other polymers. The most frequently applied method of encapsulation uses the principle of interfacial polymerization. Encapsulation in microcapsules has been extensively used to give smaller size and high efficiency to fungal biocontrol formulations (Winder, 2003; Brar, 2006).

Emulsions

It consists of liquid droplets dispersed in another immiscible liquid. Inverted emulsions are considered as good formulations due to their lower evaporation and spray drift as their external phase is oil (Brar, 2006). Studies are currently being conducted to screen a variety of oils and emulsifying agents in order to improve initial inverted emulsion formulations for biopesticides (Verner, 2007).

Suspension concentrate (SC)

SC is a mixture of a finely ground, solid active ingredient dispersed in a liquid phase, usually water. Because they are water-based, they offer many advantages, such as ease of pouring and measuring, safety to the operator and the environment, and economy. Therefore they are becoming a very popular type of formulation (Woods, 2003; Knowles, 2005).

Ultra low volume liquids (UL)

UL are formulations with very high concentration of active ingredient which is extremely soluble in crop-compatible liquid (ultra low volume liquid). UL liquid biopesticides can be formulated in a similar way using a suspended biocontrol agent as an active ingredient (Woods, 2003).

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