



E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(2): 506-510
Received: 14-01-2019
Accepted: 18-02-2019

Anusha BG

a) University of Agricultural Sciences, Raichur, Karnataka, India

b) International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Telangana, India

Naik MK

a) University of Agricultural Sciences, Raichur, Karnataka, India

b) University of Agricultural and Horticultural Sciences, Shivamogga, Karnataka, India

Gopalakrishnan S

International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Telangana, India

Amaresh YS

University of Agricultural and Horticultural Sciences, Shivamogga, Karnataka, India

Gururaj S

University of Agricultural Sciences, Raichur, Karnataka, India

Correspondence**Anusha BG**

a) University of Agricultural Sciences, Raichur, Karnataka, India

b) International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Telangana, India

A review on the biological control of plant diseases using various microorganisms

Anusha BG, Naik MK, Gopalakrishnan S, Amaresh YS and Gururaj S

Abstract

The excessive use of chemical pesticides to confront pests causes environmental pollution. Furthermore, plant pathogens resist chemical pesticides. When such pathogens accumulate in plants or soil, they will cause harmful effects on humans. Biological control is an alternative method that reduces or terminates the use of chemical compounds in agriculture. Biological control is also carried out by microorganisms. Bacteria are the main group of these microorganisms. Due to the extensive presence in the soil, tolerating changes in temperature, pH, and salinity as well as producing resistant endospore-forming species, *Bacillus* bacteria are used in biological control of soil. *Bacillus* species are often found in soil and rhizosphere. These bacteria help with the control of plant pathogens by producing siderophore, secretion of enzymes, production of antibiotics and inducing systemic resistance. In this study, various biological control mechanisms which are carried out by microorganisms have been reviewed.

Keywords: Metabolite, *Bacillus*, *Rhizoctonia bataticola*

Introduction

The increasing use of chemicals as pesticides to eliminate plant pathogens has provided effective solutions in agriculture. However, due to the fact that the excessive use of these chemicals such as thiabendazole and o-phenylphenol causes environmental pollution, and as plant pathogenic agents are quickly become resistant to chemical pesticides and considering the high price of pesticides and their accumulation in plants or soil which has harmful effects on humans, extensive researches are being conducted in the world to replace this method with more recent methods to confront fungicidal resistant pathogens. Since the late 1900s, scientists have made great efforts to use natural antagonisms of terricolous microorganisms to protect plants. Some bacteria exhibit activities against pathogens such as pathogenic fungi due to the ability to produce anti-microbial compounds such as antifungal lipopeptides and some antibiotics. For a long time, aerobic gram-negative bacteria, especially *Pseudomonas* spp, have been widely studied as biological control agents. Despite the desired characteristics of *Pseudomonas* to play a role in biological control, its main weakness as a biocontrol agent is the inability to produce spores, which has recently led to greater attention to spore-forming *Bacillus* spp. ^[1].

Agricultural waste is considered one of the most important agricultural issues for many reasons, including not observing the correct principles of harvesting, moving, transporting and storing such products. Losses and degradation caused by the action of microorganisms after harvesting result in significant economic losses in the agricultural sector. Plant-fungal diseases are one of the most important issues in agriculture and food production in the world. It is estimated that the crop losses due to plant diseases in the Western countries are 25% and in developing countries is about 50%. One hundred and one third of these damages are due to fungal diseases ^[2].

Citrus is one of the most important fruits of the world in terms of economic value, and as before reaching the market and the customer; these fruits are stored, in this time interval, they are more sensitive to post-harvest damages and are exposed to stressed aerobic and anaerobic soil conditions ^[3]. Some fungi, especially *Penicillium* species (*P. digitatum*, *P. italicum*), are involved in post-harvest losses ^[4, 5]. Other citrus pathogenic fungi include the *Aspergillus* and *Alternaria*. *Aspergillus* is the most common environmental fungi and can be isolated from citrus fruits, vegetables, tomatoes, corn, pistachios, etc. In citrus, *Aspergillus niger* citrus produces brown rot and *Aspergillus flavus* creates albinism (virescence). *Alternaria* is a species of an incomplete fungus that causes brown to black and fairly soft rot in the lemon. *Alternaria alternata* is one of the most important citrus pathogens in the world ^[6]. The primary method for controlling the fungal damage to fruits and other agricultural products is the use of chemical fungicides, some of which are not suitable

for the treatment of these products, and some have been eliminated due to the possibility of having toxic agents [7]. Recently, the use of biological control agents, especially bacteria, has attracted a lot of attention due to the ability of some species to suppress different plant diseases and the possibility of combining with other control methods [8]. Therefore, various sources of antibiotic production are screened, among which *Bacillus* especially is an important alternative to extract antibiotics and their industrial production. The soil is considered one of the main sources of microbes and naturally is a habitat for a large group of bacteria that is the source of bioactive products with diverse pharmacological activities. Soil bacteria, especially the *Bacillus* species, grow rapidly and are characterized by synthesis of secondary metabolites with significant variations in structure and performance and as biocontrol plants (fungicides, bactericides, and fertilizers), probiotics and pathogens [9, 10].

Therefore, in the growing trend of the use of secondary bacterial metabolites in medicine and industry, it seems that in the biological control of fungal diseases of the soil, the useful microorganisms of rhizosphere or plant probiotics are appropriate alternatives for the first line of defense against these pathogens. So, the present research studied various plant pests to control plant diseases and described the biological methods such as using antifungal extracts of bacteria to confront such diseases.

Biological control in plant diseases management

An increase in population and consequently an increase in demand for food as well as the limitation of agricultural land use have led to an increase in crop yields per unit area. In order to achieve sustainability, the need for sustainable agriculture should be felt. Achieving a sustainable lifestyle requires reducing the use of chemical pesticides and applying biological methods against pests and diseases [13]. As the excessive use of chemicals in agriculture causes environmental pollution, throughout the world, comprehensive researches have been conducted to replace this method with newer methods to confront fungal resistant pathogens. An essential solution is the utilization of the activity and function of microorganisms [14]. Biological control reduces the effects of pesticide use in the long term and makes a balance between harmful plant pathogens and their natural enemies. In this regard, antagonistic bacteria and fungi are widely used to control plant diseases [15].

Biological control mechanisms

Because biological control can result from many different types of interactions between organisms, researchers have focused on characterizing the mechanisms operating in different experimental situations. In all cases, pathogens are antagonized by the presence and activities of other organisms that they encounter. Direct antagonism results from physical contact and/ or a high-degree of selectivity for the pathogen by the mechanism(s) expressed by the BCA(s). In contrast, indirect antagonisms result from activities that do not involve sensing or targeting a pathogen by the BCA(s). In hyperparasitism, the pathogen is directly attacked by a specific BCA that kills it or its propagules. In general, there are four major classes of hyperparasites: obligate bacterial pathogens, hypoviruses, facultative parasites, and predators. *Pasteuria penetrans* is an obligate bacterial pathogen of root-knot nematodes that has been used as a BCA. Hypoviruses are hyperparasites. 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. Other hyperparasites attack plant-pathogenic nematodes during different stages of their life cycles (e.g. *Paecilomyces lilacinus* and *Dactylella oviparasitica*). Some BCAs exhibit predatory behavior under nutrient-limited conditions. However, *Trichoderma* produces a range of enzymes that are directed against cell walls of fungi.

Antibiotics are microbial toxins that can, at low concentrations, poison or kill other microorganisms. In some instances, antibiotics produced by microorganisms have been shown to be particularly effective at suppressing plant pathogens and the diseases they cause. To be effective, antibiotics must be produced in sufficient quantities near the pathogen to result in a biocontrol effect. However, the effective quantities are difficult to estimate because of the small quantities produced. The relative importance of antibiotic production by biocontrol bacteria has been demonstrated, where one or more genes responsible for biosynthesis of the antibiotics have been manipulated. Several biocontrol strains are known to produce multiple antibiotics which can suppress one or more pathogens. The ability to produce multiple classes of antibiotics, that differentially inhibit different pathogens, is likely to enhance biological control.

Parasitism

It is a coexisting relation in which two microorganisms that are not evolutionally related to each other live together for a long period of time, and as a result of this kind of relation, usually one of these two types of microorganisms which is physically smaller and is called the parasite, benefits from the other one which is partially damaged and is called the host. The most direct type of competition in a biological control is hyperparasitism in which pathogenic parasites are used to eliminate a pathogen. The most direct type of competition in biological control is hyperparasitism, in which obligate parasites are used to eliminate the same plant pathogen [13]. Biological control also means a reduction in nematode damage by organisms antagonistic to nematodes through the regulation of nematode populations and/or a reduction in the capacity of nematodes to cause damage, which occurs naturally or is accomplished through the manipulation of the environment or by the mass introduction of antagonists [16]. Biological systems can improve the soil consistency and maintain natural soil flora. Microorganisms from each rhizosphere soil develop the plant resistance against pathogens [17].

Competition

It is a kind of mutual coexistence between two living microorganisms, which occurs when different microorganisms within a population attempt to achieve something similar. The target may be a place or food [13].

Predation

The primarily refers to animals that at the higher stage of nourishment and in the macroscopic world are predations [13].

The primarily refers to animals that at the higher stage of nourishment and in the macroscopic world are predations ^[13].

Antagonism

It is the negative effect that a microorganism has on another one. This is a one-way process and is due to the production of certain compounds such as antibiotic or bacteriocin produced by a microorganism ^[13].

Microorganisms affecting biological control

Fungi

Trichoderma and *Gliocladium* are fungi that biologically control fungal pathogens ^[18]. They suppress the plant fungal pathogens through different mechanisms and by producing antimicrobial compounds such as antibiotics with a complex structure like gliovirin and gliotoxin.

Viruses

Viruses are used for biological control of insect-borne plant pathogens. Baculoviridae, Poxviridae, Reoviridae, Iridoviridae, Parvoviridae, Picornaviridae and Rhabdoviridae are used for an effective biological control ^[19].

Bacteria

a. *Pseudomonas* species

Pseudomonas spp has many characteristics that make them biocontrol, such as colonization and proliferation within the plant, competition with other microorganisms, adaptation to environmental stresses, and the production of a wide range of active biometabolites such as antibiotics, sydropores, volatile substances, and growth stimulant compounds ^[20]. Due to this, such bacteria are used to suppress plant pathogens. Among the most common *Pseudomonas* species which have a role in biological control, we can name *P. fluorescens*, *P. chlororaphis*, *P. aureofaciens*, *P. putida* and among non-pathogenic species of this bacteria we can refer to *P. syringae* ^[21]. These species are used in the suppression of plant diseases such as Take-all (Take-all is a plant disease affecting the roots of grass and cereal plants in temperate climates caused by the fungus *Gaeumannomyces graminis* var. *tritici*. All varieties of wheat and barley are susceptible) and Fire Blight (caused by the bacterium *Erwinia amylovora*, is a common and frequently destructive disease of pome fruit trees and related plants). Pear (*Pyrus species*) and quince (*Cydonia species*) are extremely susceptible. Apple, crabapple (*Malus species*), and firethorns (*Pyracantha species*) also are frequently damaged ^[22].

b. *Bacillus* species

The *Bacillus* spp. was discovered in 1872 by Chon. Gram-positive *Bacillus* spp. are rod-shaped, optional aerobic or anaerobic, catalase-positive, and have peripheral cilium. These microorganisms have a low chemoorganotrophic metabolism (chemoorganotrophs are organisms which oxidize the chemical bonds in organic compounds as their energy source). The most important characteristic of the members of this species is the production of endospore, which helps with the durability of bacteria in the nature and can last for a very long time, perhaps millions of years ^[23].

A review on the role of *Bacillus* spp. In biological control of plant diseases

Bacilli associated with the plant are known as plant parasites, saprophytes, and biological control agents. Three species of *Bacillus* are considered as plant parasites. These species

include *Bacillus megaterium* pv. *cerealis* causing White Blotch Incited in Wheat, *B. circulans* which causes the death of date seedlings and *Bacillus polymyxa* causing blight tomato. Most *Bacillus* species are harmless saprophytes ^[24].

Bacillus species are good candidates for biological control due to their specific characteristics. First, they produce anti-fungal and anti-bacterial antibiotics. Their second feature is the ability to produce spores, due to the same characteristic, it is easy to be formulated because comparing with tube cells it survives longer. It is worth mentioning that, rhizospheric microbes have the immense potentiality to synthesize and release various compounds that are regulating plant growth as well as the physical and chemical texture of the soil. Thus, the charcoal-based formulation of *Bacillus* species can be used for plant growth promoting the activity of various crops ^[25]. Another feature is their permanent presence (because they are part of the general microflora of the soil) in the soil ^[26]. Among the activities of antibiotics produced by this bacterium, one can name their direct effect on fungi, competition with microorganisms which attack root systems. *Bacillus* bacteria seem to be appropriate solutions to control vascular pathogens due to their ability to colonize the internal organs of the plant. The results of the studies conducted by Kim *et al.* ^[27] indicate that *Bacillus* spp controls wheat diseases. *B. subtilis* inhibits germination of plant pathogen spores, causes degradation or disruption of their germ tube growth and interferes with the attachment of pathogen to the plant.

Bacillus

Bacillus species include gram-positive bacteria which are rod-shaped and often move. These bacteria can produce products such as ethanol, H₂, acetone, acetic, formic, lactic, and succinic acids by fermentation of glucose. *Bacillus subtilis* YM 10-20 can produce compounds which resist against fungi ^[28]. Among these compounds, one can point to the extract from the etorin A, which was first extracted from *Bacillus circulans*. This compound is a cyclic lipopeptide that has an inhibitory effect on fungal growth while has a little toxic effect on humans. Thus, it is used in the treatment of fungal infections in humans and animals. Other *Bacillus* species that are able to produce Euthorin A is *B. amilolycophasins*. This species of *Bacillus* is used as a biological controller against plant pathogenic fungi ^[26]. A study on the antifungal metabolites of *Bacillus* species shows that these metabolites are resistant to temperature and pH changes and do not lose their antifungal effect ^[29].

B. subtilis is also able to produce a metabolite called surfactin, which is a strong biosurfactant and has an antifungal activity. A compound which is called fusaricidin A, also has anti-fungal properties and is obtained from *B. polymyxa*. Fusaricidin A acts against both fungi and gram-positive bacteria.

Bacillus

A source of biologically active molecules Most *Bacillus* species are considered as microbial factories which produce a wide range of biologically active molecules that have inhibitory properties towards the growth of pathogens ^[30]. One of the most common and well-known microorganisms is *B. subtilis*. An average of 4-5% of its genome is used for antibiotic synthesis and can produce different antimicrobial compounds with different structures ^[31]. Among the antimicrobial compounds, the potential of cyclic lipopeptides such as Surfactin, etorin, and fengycin for biotechnology and

pharmacy applications is well-known for their surfactant properties^[32].

Characteristics and biological effects of non-protein toxins from *Bacillus* species

Many microbial peptides are synthesized by peptide synthetases which are multienzyme complexes^[36]. About 4% to 7% of the genomes of *Bacillus subtilis* and *B. amyloliquefaciens* are used to produce biologically active compounds^[37]. Surfactin, Lichenysin A, Fengycin, Tricocidine, and Bacitracin mycosubtilin are produced by Non-Ribosomal Peptide Synthesis^[38]. *Bacillus* species produce a wide variety of antibacterial, antiviral and antimicrobial compounds, some of which are toxic for eukaryotic cells. *Bacillus* toxins are associated with various chemical groups whose structure consists of cyclic lipopeptides, cyclic peptides, phospholipid oligopeptides, low-molecular-weight compounds with NH₂ (amino group) functional groups as side chains (R group which determines the properties of each amino acid) and a cationic functional group of the sugar derivative. The peptides have a molecular weight between 500-4000 daltons with the average molecular mass of 1000 daltons. Most of the non-protein extracts of *Bacillus* species discovered before the 1980s while their toxic properties were identified later^[39]. *Bacillus* strains produce compounds that in different ways change the function of biological membranes. Some compounds affect the integrity of the plasma membrane (such as Surfactin and Lichenysin A) and may cause electrolyte leakage, loss of cell contents, and cell death^[40]. The ionophoric materials (such as Gramicidin S, Gramicidin A) form ion channels or act as ion carriers (Ibid). Such substances change the fluidity of the plasma membrane or the organelles membranes. Protecting transmembrane electrical potential, as well as the proper ionic concentration of cells or organelles, are vital for cellular functions. Many biochemical functions are regulated by the ion flow in the membranes and the change in the structure of the membrane proteins (Ibid). The change in membrane potential changes the structure of membrane proteins such as Na⁺/K⁺ channels which may cause cell death^[30].

Conclusion

For many years, agricultural waste has been considered as an important issue due to numerous reasons, including non-compliance with the principles of harvesting, moving, transporting and maintaining such products. Losses and degradation resulted from microorganism activities after harvesting causes significant economic losses to agricultural products. As health is related to the type of diet, the human being who is the main consumer of agricultural products is made to seek foods free from pesticide residues, toxins, and harmful microorganisms. As chemical pesticides are expensive, threaten both human health and environment, and some extracts of pathogens are resistant to them, recently, biological control agents are used to fighting plant pathogens. Bacteria, as a type of biological control agents, have attracted a great deal of attention due to the ability of some species to suppress different plant diseases through different mechanisms and the possibility of combining with other control methods.

Terricolous and non-pathogenic bacteria with the antagonistic ability of plant pathogenic fungi and disease prevention are an appropriate alternative to chemical fungicides and are one of the most important factors in establishing and sustaining agricultural systems. Biological control reduces the effects of

pesticide in the long run, leads to a balance between harmful plant pathogens and their natural enemies. In this regard, antagonistic bacteria and fungi are widely used to control plant diseases. In comparison with chemical controls, biological control is a healthier and safer control since, unlike some toxic substances, microorganisms are not stored and accumulated in food chains. Biological control is carried out through mechanisms such as competition, antibiosis, predation, and parasitism.

References

- Mavrodi DV, Yang MM, Mavrodi OV. Management of soilborne plant pathogens with beneficial root-colonizing *Pseudomonas* (Chapter 7). *Adv PGPR Res*, 2017, 147.
- Gohel V, Singh A, Vimal M. Bioprospecting and antifungal potential of chitinolytic microorganisms. *Afr J Biotechnol*. 2006; 5:54-72.
- Usall J, Ippolito A, Sisquella M. Physical treatments to control postharvest diseases of fresh fruits and vegetables. *Postharvest Biol Technol*. 2016; 122:30-40.
- Barkai-Golan R. *Postharvest diseases of fruits and vegetables: Development and control*. Elsevier, 2001.
- Eckert JW, Eaks IL. *Postharvest disorders and diseases of citrus fruits*. The citrus industry. 1989; 5:179-260.
- Walderhaug M. *Foodborne pathogenic microorganisms and natural toxins handbook*. Bad Bug Book. 2009, 5.
- D'Aquino S, Chessa I, Inglese P. Increasing cold tolerance of cactus pear fruit by high temperature conditioning and film wrapping. *Food Bioprocess Tech*. 2017; 10:1466-1478.
- Arrebola E, Jacobs R, Korsten L. Iturin A is the principal inhibitor in the biocontrol activity of *Bacillus amyloliquefaciens* PPCB004 against postharvest fungal pathogens. *J Appl. Microbiol*. 2010; 108:386-395.
- Mongkolthanaruk W. Classification of *Bacillus* beneficial substances related to plants, humans and animals. *J Microbiol. Biotechnol*. 2012; 22:1597-1604.
- Amin A, Khan MA, Ehsanullah M. Production of peptide antibiotics by *Bacillus* sp: GU 057 indigenously isolated from saline soil. *Braz J Microbiol*. 2012; 43:1340-1346.
- Stirling GR. Biological control of plant-parasitic nematodes. In *Diseases of nematodes*. CRC Press, 2017, 103-150.
- Fravel DR, Keinath AP. Biocontrol of soil borne plant pathogens with fungi. In *The rhizosphere and plant growth*. Springer, Dordrecht. 1991, 237-243.
- Bonning BC. Editorial overview: Parasites/Parasitoids/Biological control: Virus-insect interactions: Progress and pitfalls. *Curr Opin Insect Sci*. 2015; 8:7-9.
- Stockwell VO, Stack JP. Using *Pseudomonas* spp. for integrated biological control. *Phytopathology*. 2007; 97:244-249.
- Siddiqui ZA, Khan M. Biofilm formation by *Pseudomonas* spp. and their significance as a biocontrol agent. *Biofilms in Plant and Soil Health*. 2017; 14:69.
- Agaras BC, Scandiani M, Luque A. Quantification of the potential biocontrol and direct plant growth promotion abilities based on multiple biological traits distinguish different groups of *Pseudomonas* spp. isolates. *Biol Control*. 2015; 90:173-186.
- Zimina MI, Sukhih SA, Babich OO. Investigating antibiotic activity of the genus *Bacillus* strains and properties of their bacteriocins in order to develop next-generation pharmaceuticals. *Foods Raw Mater*, 2016, 4.

18. Kumar RM, Kaur G, Kumar A. Taxonomic description and genome sequence of *Bacillus campisalis* sp. nov., A member of the genus *Bacillus* isolated from a solar saltern. *Int J Syst Evol Microbiol.* 2015; 65:3235-3240.
19. Pahari A, Pradhan A, Maity S. Carrier based formulation of plant growth promoting *Bacillus* species and their effect on different crop plants. *Int J Curr Microbiol App Sci.* 2017; 6:379-385.
20. Balouiri M, Bouhdid S, Harki EH. Study on the effect of the antifungal extract from *Bacillus* sp. on the physicochemical properties of *Candida albicans*. *Res J Microbiol.* 2015; 10:214.
21. Kim DS, Cook RJ, Weller DM. *Bacillus* sp. L324-92 for biological control of three root diseases of wheat grown with reduced tillage. *Phytopathology.* 1997; 87:551-558.
22. Chitarra GS, Breeuwer P, Nout MJ. An antifungal compound produced by *Bacillus subtilis* YM 10-20 inhibits germination of *Penicillium roqueforti* conidiospores. *J Appl Microbiol.* 2003; 94:159-166.
23. Sansinenea E, Ortiz A. Secondary metabolites of soil *Bacillus* spp. *Biotechnol Lett.* 2011; 33:1523-1538.
24. Passari AK, Mishra VK, Saikia R. Isolation, abundance and phylogenetic affiliation of endophytic actinomycetes associated with medicinal plants and screening for their *in vitro* antimicrobial biosynthetic potential. *Front Microbiol.* 2015; 6:273.
25. Kumar KS, Haritha R, Mohan YS. Screening of marine actinobacteria for antimicrobial compounds. *Res J Microbiol.* 2011; 6(4):385-93.
26. Huszcza E, Burczyk B. Surfactin isoforms from *Bacillus coagulans*. *Zeitschrift für Naturforschung.* 2006; 61:727-733.
27. Behera SS, Ray RC. Solid state fermentation for production of microbial cellulases: Recent advances and improvement strategies. *Int J Biol Macromol.* 2016; 286:656-669.
28. Heidari A. Genomics and proteomics studies of zolpidem, necopidem, alpidem, saripidem, miroprofen, zolimidine, olprinone and abafungin as anti-tumor, peptide antibiotics, antiviral and central nervous system (CNS) drugs. *J Data Mining Genomics Proteomics,* 2016, 7-25.
29. Stein T. *Bacillus subtilis* antibiotics: Structures, syntheses and specific functions. *Mol Microbiol.* 2005; 56:845-857.
30. Koumoutsis A, Chen XH, Henne A. Structural and functional characterization of gene clusters directing non ribosomal synthesis of bioactive cyclic lipopeptides in *Bacillus amyloliquefaciens* strain FZB42. *J Bacteriol.* 2004; 186:1084-1096.
31. Sansinenea E. Discovery and description of *Bacillus thuringiensis*. In *Bacillus thuringiensis Biotechnology,* Springer, Dordrecht, 2012, 3-18.
32. Quadros CP, Duarte MC, Pastore GM. Biological activities of a mixture of biosurfactant from *Bacillus subtilis* and alkaline lipase from *Fusarium oxysporum*. *Braz J Microbiol.* 2011; 42:354-361.