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Microbial metabolites for development of ecofriendly agrochemicals

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ABSTRACT

Owing to the enhanced public concern regarding the further use of synthetic chemicals as pesticides, there is a pressing need to explore greener, innovative alternatives to fulfill the ever growing food demand. Microbes are rich source of a variety of chemical compounds leading to development of drugs/pesticides. The huge biodiversity of microorganisms and their ecological interactions with other organisms is an attractive resource for harnessing novel signal molecules, which could help in discovery and development of next generation of agrochemicals. This review examines the possible role of these microbial natural products in development of biorational agrochemicals, which would have least impact on the environment and would also be benign to other non-target organisms.

Key words: Allelochemicals, biorational agrochemicals, crop protection, microbial secondary metabolites, natural products, pests, phytotoxins, toxins.

1. INTRODUCTION

In past hundred years, agriculture has become too intensive to meet the food demand of world population. Apart from mechanization, the discovery and use of synthetic chemical moieties have played a key role in enhancing the crop yields and minimising the crop losses from pests (weeds, plant pathogens, insects nematodes). It is expected that the demand for cereals for food and animal feed use would touch 3 billion tonnes in 2050. Projections also indicate the need to increase the overall plant production by 70% to feed a population of 9.1 billion in 2050 (66)

The reliance on synthetic agrochemicals to meet the growing food demands has led to the environment and health hazards. Synthetic agrochemicals pose risk to non-target organisms including humans leading to societal and scientific concern globally regarding their further use. Residual toxicity of these xenobiotics has resulted in high incidences of cancer, hormonal and immunological disorders and allergies apart from the effects on reproductive ability. Owing to the residual toxicity of xenobiotics, there is also a shift in pest management, wherein the emphasis is on suppression of pest population to sub-economic and sub-lethal levels rather than complete eradication as practiced previously. With the growing demand of organically grown food, suitable non-hazardous and innovative alternatives are being explored (14). Currently the crop protection groups address a complex set of questions in mind, while designing the pesticides viz., What form should the crop protection agent take to prevent harm to the user, soluble in water, sprayed without difficulty, controls the pest in crops at lowest concentration and do not harm the environment (9,13).

Antagonism and allelopathy are the main interactions which has attracted the attention of researchers for designing suitable replacements of current synthetic chemicals. Rice (58) defined allelopathy as the chemical interaction of microbial and plant secondary metabolites with organism to affect the growth and development of biological systems. The microbial and plant secondary metabolites participating in allelopathy are called allelochemicals.

Microbes are ubiquitous and display various interactions with other living organisms mediated by a myriad of chemical interactions that exhibit diverse biological activities. A wide range of pharmacophores have been isolated from microbes and developed into drugs like streptomycin (*Streptomyces griseus*), penicillin (*Penicillium chrysogenum*), bacitracin (*Bacillus subtilis*) and lovastatin (*Monascus ruber*). Dedicated efforts similar to pharmaceutical screening programmes are needed to explore the pesticidal potential of microbial secondary metabolites for their direct or indirect effects to develop biorational and ecofriendly agrochemicals. Microbial secondary metabolites or natural products have relatively shorter environmental life spans, are biodegradable, hence pose lower threat of residual toxicity than conventional halogenated chemical structures.

There is an enormous microbial diversity, with microbes being isolated from all environments, including unique ecological niches such as the gut of termites and fruit flies. Such organisms exist as endophytes inside woody and non-woody plants and in marine organisms. Thus there is a huge potential for screening of new secondary metabolites with applications in agrochemical and pharmaceutical industry. There are over 23,000 known secondary metabolites, of which 42% are produced by fungi, 42% by actinomycetes and 16% by other bacterial species. This review surveys microbial secondary metabolites with efficacy in pest management (i.e. weeds, plant pathogens, insects and nematodes). It also highlights the importance of microbial secondary metabolites in discovery of new pesticidal modes of action.

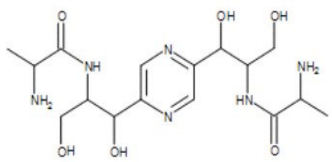
2. BACTERIA

Numerous bacterial secondary metabolites have been isolated and tested for their use as agrochemicals. Many of these posess herbicidal, insecticidal or fungicidal activity.

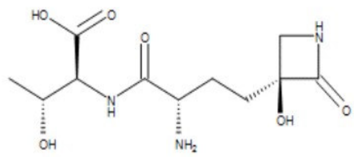
2.1. INSECTICIDES

(i). **Bt-Toxins**: The most effectively exploited bacterial secondary metabolites as bio-insecticides are the endotoxins produced by *Bacillus thuringensis* and related species. These are collectively called the Bt-toxins. Transgenic plants expressing the insecticidal bacterial toxins are used to control butterflies and moths (Lepidoptera), flies and mosquitoes (Diptera) and beetles (Coleoptera).

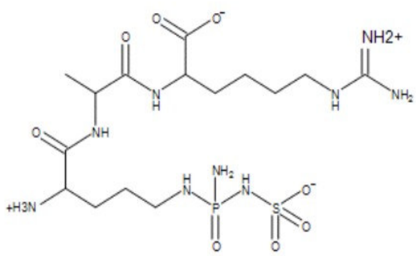
(ii). **Diabroticin A:** Diabroticin A **(I)** is a polar insecticide produced by *Bacillus cereus* and *Bacillus subtilis* which is active against Southern corn rootworm (*Diabrotica undecimpunctata*) through diet (60).



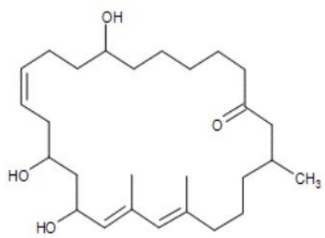
I Diabroticin



II Tabtoxin



III Phaseolotoxin



IV Macrolactin A

Figure 1. Bacterial secondary metabolites as agrochemicals

2.2 HERBICIDES

(i). **Tabtoxin:** The phytotoxic potential of bacterial secondary metabolites was found with the discovery of wildfire toxin also called Tabtoxin (**II**) produced by *Pseudomonas syringae* var. *tabaci* responsible for the wildfire disease of tobacco. Tabtoxin inhibits the glutamine synthetase activity.

(ii). **Phaseolotoxin:** *Pseudomonas sp.* has also produced some broad spectrum or non-host specific phytotoxins like phaseolotoxin **(III)**. Phaseolotoxin is produced by *Pseudomonas syringae* pv. *phaseolicola* and exhibits broad range of

Table 1. Biorational agrochemicals produced by bacteria

Allelochemical	Source	Bioactivity against organisms						Ref.
		Crops	Weeds	Fungi	Bacteria	Nematodes	Insects	
Diabrocitin	*Bacillus cereus*	-	-	-	-	-	+	60
Iturin	*Bacillus sp. sunhua*	-	-	+	+	-	-	30
Macrolactin	*Bacillus sp. sunhua*	-	-	+	+	-	-	30
Phaseolotoxin	*P. syringae var. phaseolicola*	-	+	-	-	-	-	5
Syringomycin E	*Pesudomonas syringae ESC 10/11*	-	-	+	-	-	-	7
Tabtoxin	*Pseudomonas syringae var tabaci*	+	+	-	-	-	-	32

Table 2. Actinomycetes as sources of ecofriendly agrochemicals

Allelochemical	Source	Bioactivity against organisms						Ref.
		Crops	Weeds	Fungi	Bacteria	Nematodes	Insects	
Albucidin	*Streptomyces albus subsp. chlorinus NRRL B24108*	-	+	-	-	-	-	29
Anisomycin (Methoxyphenone)	*Streptomyces griseolus*	-	+	-	-	-	-	35
Avermectin B1a/B1b	*Streptomyces avermitilis*	-	-	-	-	-	+	38
Bialaphos(Herbiace®)	*Streptomyces hygroscopicus/ Streptomyces viridiochromogenes*	+	+	+	-	-	-	48
Blasticidin- S	*Streptomyces griseochromogenes*	-	-	+	-	-	-	23
Borrelidine	*Streptomyces sp.-neau-D50*	-	-	+	-	-	-	76
Herbimycin	*Streptomyces hygroscopicus AM3672*	-	+	-	-	-	-	37
Kusagaemycin	*Streptomyces kusagaensis*	-	-	+	-	-	-	69
Milbemycin A3/A4	*Streptomyces subsp.aureolacrimosus*	-	-	-	-	-	+	55
Oxytetracycline	*Streptomyces rimosus*	-	-	+	+	-	+	21
Pyrizadocidin	*Streptomyces # 620061*	-	+	-	-	-	-	25
Spinosyn A/D	*Saccharopolyspora spinosa*	-	-	-	-	-	+	58
Tartrolone C	*Streptomyces sp. CP1130*	-	-	-	-	-	+	44

Table 3. Pesticidal compounds produced by Cyanobacteria

Allelochemical	Source	Bioactivity against organisms						Ref
		Crops	Weeds	Fungi	Bacteria	Nematodes	Insects	
Cyanobacterin	*Scytonema hofmanii*	-	+	-	-	-	-	26
Dihydroactiniodiolide	*Phormidium angustissium*	-	-	-	-	-	-	10
Eremophilane	*Claothrix sp. PCC 7507*	-	-	-	-	-	-	31
Hapalindole L	*Fischerella ATCC 43239*	-	-	-	-	-	+	3
12-epi-Hapalindole C		-	-	-	-	-	+	3
12-epi- Hapalindole J isonitrile		-	-	-	-	-	+	3
2(R), 5(R)-bis-Hydroxymethyl)-3(R), 4(R)-dihydroxypyrrolidine (DMDP)	*Cylindrospermum licheniforme*	-	-	-	-	-	+	39

phytotoxic activity by inhibiting the ornithine carbamoyl transferase (OCT), which regulates the arginine synthesis.

(iii). **Coronatine:** Coronatine produced by *Pseudomonas coronafacience* has structural and functional similarity to jasmonates and jasmonic acid-isoleucine (JA-Ile) (32). It seizes the jasmonate controlled pathways in host being a jasmonate mimic, thereby, deregulating many important processes (5). Coronatine exhibits a typical chlorotic pattern in the developing tissues of plant.

2.3 FUNGICIDES:

(i). **Macrolactin A:** Macrolactin A **(IV)** and iturin A produced by *Bacillus* sp. *sunhua* inhibited the potato scab pathogen *Streptomyces scabies* and is also fungicidal to *Fusarium oxysporum* causing dry rot disease (30).

(ii). **Syringomycin E:** Syringomycin E from *Pseudomonas syringae* ESC 10 /11 controls the citrus green mold *Penicillium digitatum* responsible for huge economic losses (7).

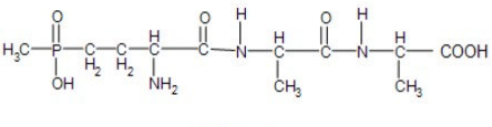
3. ACTINOMYCETES

Amongst the soil microbes, actinomycetes occupy important position, as they produce wide spectrum of biologically active substances already commercialized as pharmaceuticals and agrochemicals. Today actinomycetes mainly the *Streptomycetes* group, provide bioactive agents used as herbicides, fungicides or bactercides, insecticides and acaricides.

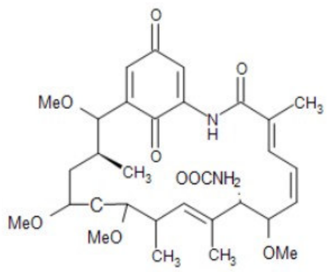
3.1. HERBICIDES

As actinomycetes are major group of soil inhabiting microbes and exist in all types of soils, they have developed inherent mechanisms to fight stressful conditions by producing the secondary metabolites which could be exploited as agrochemicals. As plants also produce many allelochemicals in rhizosphere, these microbes produce an array of metabolites, these with plant produced inhibitory compounds may be exploited as herbicides. Some promising herbicides from Actinomycetes are: Bialaphos, Herbimycin and Pyrizadocidin.

(i). **Bialaphos**: The herbicidal potential of actinomycetes came into limelight with the discovery of bialaphos **(V)** from *Streptomyces hygroscopicus* and *Streptomyces viridiochromogenes* in 1978 (48). Bialaphos is a tripeptide ((L-2-amino–4-[(hydroxy)(methyl)phosphinoyl]–butyryl-L-alanyl–L-alanine) herbicide produced through fermentation by Meiji Seika and sold as Herbiace®. The other common names of bialaphos are bilanafos, phosphinothricyl- alanyl-alanine and phosphinothricin-alanyl- alanine. Bialaphos in plants is converted into actual phytotoxin, phosphinothricin by hydrolytic cleavage of two alanyl groups and irreversibly inhibits the GS (67). Bialaphos is post emergence herbicide in vines, apples, brassicas or on uncultivated land for post- emergence control of annual weeds in crops (11). Chemically synthesized version of phosphinothricin is glufosinate ammonium introduced by Hoechst (now Bayer CropScience) as a non- selective herbicide sold under the trade names Basta®, Ignite® and Liberty®. Bilanafos and glufosinate are biodegradable and non-toxic to mammals and non-target organisms. They are regarded as low environmental impact herbicides.

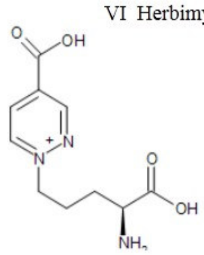


V Bialaphos

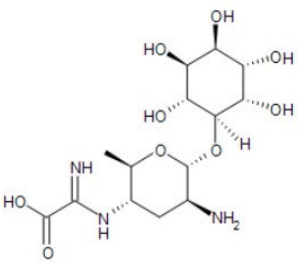


VI Herbimycin

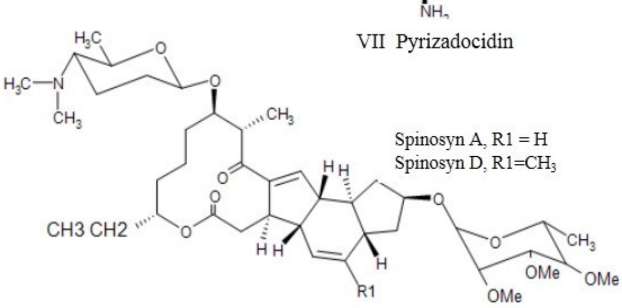
VIII Blasticidin-S



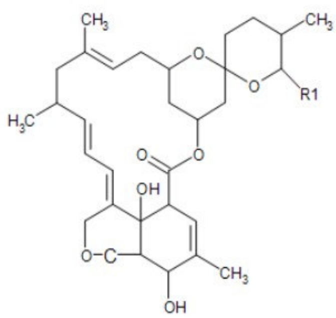
VII Pyrizadocidin



IX Kasugamycin



X Spinosad



XI Milbemycin

Figure 2. Agroactive compounds produced by Actinomycetes

(ii). **Methoxyphenone:** Methoxyphenone (3,3-dimethyl-4-methoxy benzophenone) was the first synthetic herbicide developed (36) on template of microbial natural product anisomycin isolated from *Streptomyces griseolus*. Both anisomycin and methoxyphenone are excellent herbicides against barnyardgrass and crabgrass (*Digitaria* sp.) but are non-toxic to *Brassica rapa* L. (turnip) or *Lycopersicon esculentum* (35).

(iii). **Herbimycin :** Herbimycin **(VI)** is a benzaquinoid ansamycin antibiotic with potential herbicidal activity produced by *Streptomyces hygroscopicus* AM3672. Herbimycin possess broad spectrum herbicidal activity (i.e effective against both monocot and dicot weeds). It both a pre- and post-emergence herbicide (29).

(iv). **Pyrizadocidine:** Pyrizadocidin **(VII)** is produced by the *Streptomyces* #620061 isolated from a Honduran soil sample (25). Pyridazocidin has herbicidal activity by rapid induction of chlorosis and necrosis in weeds. The mode of action of pyrizadocidin is reversible oxidation/reduction linked photosynthetic electron transport (Mehler's reaction). Albucidin isolated from *Streptomyces albus* subsp. *chlorinus* NRRL B-24108 is phytotoxic and induces chlorosis and bleaching (29). Post emergence activity of albucidin is broad spectrum. The mode of action of albucidin may be similar to hydantocidin, which inhibits the adenylosuccinate synthetase.

3.2. FUNGICIDES

Streptomyces species also produce an array of secondary metabolites with applications as fungicides and bactericides. Actinomycetes and Streptomyces species exhibiting fungicidal activity to fungi have been screened and developed into commercial fungicides.

(i). **Blasticidin-S (VIII):** It is a soil actinomycete (*Streptomyces griseochromogenesI*) product used to control the rice blast caused by *Pyricularia oryzae* by foliar application (23). Blasticidin-S is a contact fungicide possessing protective and curative action. Three formulations viz., dustable powder (DP), emulsifiable concentrate (EC) and wettable powder (WP) of blasticidin-S are sold under the name Bla-S by Kaken, Kumiai and Nihon Noyaku.

(ii). **Kusagamycin (IX)**: It is recommended to control rice blast caused by *Pyricularia oryzae*, leaf spot in sugar beet and celery by *Cercospora* spp. and scab in pears and apples caused by *Venturia* spp. Kasugamycin and its hydrochloride hydrate derivative was isolated from *Streptomyces kasugaensis* (69). Kasugamycin is sold as WP, DP, granules (GR) and soluble concentrates under the tradename of Kasugamin and Kasumin from Hokko. Slight phytotoxicity occurs on some crops (e.g. potatoes, rice, grapes, citrus and apples during foliar spray. An endophytic *Streptomyces* species- neau-D50 isolated from healthy roots of soybean produced borrelidin with very strong antifungal activity against *Phytophthora sojae* with EC_{50} and EC_{95} of 5.6 and 26 µg/l, respectively. These EC values are several folds lower than commercial fungicide metalaxyl, current fungicide to control *P. sojae* and thus appears as promising candidate for fungicide development (76).

3.3. INSECTICIDES

Actinomycetes are also prolific producers of insecticidal agents. The success rate of secondary metabolites of actinomycetes is much better and similar to conventional agrochemicals than their use as biological control agents. The advantage of these compounds over conventional chemicals is selective action and minimal residual effect.

(i). **Abamectin:** It is an insecticide and acaricide isolated from the fermentation broth of *Streptomyces avermitilis*. It is a mixture of avermectin B1a and avermectin B1b (38) introduced commercially first by Merck, Sharpe and Dohme Agvet and now owned by Syngenta. Abamectin controls motile forms of wide range of mites, leafminers, suckers, beetles and other insects which attack on ornamentals, cotton, citrus fruits, vegetables, potatoes etc. The target site of abamectin is γ-aminobutyric acid (GABA) receptor in insects. Commercially abamectin is sold as EC formulation under the trade names Agri-Mek, Avid, Clinch and others. The synthetic version of abamectin, emamectin benzoate, is also insecticidal and acaricidal. It comprises of two homologues emamectin B1a and emamectin B1b in a ratio 90:1. It is particularly effective against lepidopteran insects apart from control of pinewood nematodes [*Bursaphelenchus xylophilus* (Steiner& Buhrer) Nickle]. It is sold as EC and WG formulation under the trade names Proclaim, Affirm, Arise and Denim all produced by Syngenta. Both abamectin and emamectin are rapidly degraded in the soil by microorganisms (19).

(ii). **Spinosad (X):** It is a secondary metabolite of *Saccharopolyspora spinosa* Mertz &Yao isolated from soil in Caribbean (52, 68). It is a mixture of spinosyn A and spinosyn D and is used to control the wide range of caterpillars, leaf miners thrips and foliage feeding beetles. It also controls the caterpillar (*Helicoverpa zea* Boddie, *Pieris rapae* (L.), *Keiferia lycopersicella* (Walsingham), thrips (*Ceratitis capitata* (L.), *Thrips palmi* (Karny)) and beetles (*Leptinotarsa decemlineta* (Say)). Spinosad is sold as a water base suspension concentrate (SC) formulation as Tracer, Spinoace (Dow AgroScience), Entrust and Conserve. Spinosad is degraded by photolysis on soil surface, whereas inside the soil, it is degraded by microorganisms.

(iii). **Milbemycin (XI):** It is yet another insecticidal and acaricidal product isolated from the fermentation broth of *S. hygroscopicus* subsp. *aureolacrimosus* (55). A mixture of milbemycin A3 and milbemycin A4 in ratio 3:7 is commercialized by Sankyo Agro. Milbemycin controls citrus red mites, kanzawa spider mites and leaf miners in citrus fruits, tea, aubergines and protected ornamentals. The mode of action of milbemycin is inhibition of neurotransmission in insects as it causes more release of GABA its binding at receptor leading to hyperpolarisation. Milbemycin is an acaricide with contact and stomach action. EC and WP formulations of milbemycin are sold under the trade names of Milbeknock, Koromite and Matsuguard by Sankyo Agro. Synthetic analogue milbemycin oxime is used to treat various nematodes infesting pet animals such as dogs and is sold commercially as Inceptor and Sentinel.

(iv). **Tartrolone C:** It is isolated as novel insecticidal macrodiolide produced by *Streptomyces* sp. CP1130 (44).

3.4. ANTIBIOTICS

Antibiotics are used to treat human diseases and also to control plant diseases caused by bacteria and fungi.

(i). **Oxytetracycline:** The antibiotic oxytetracycline is used to control fire blight caused by *Erwinia amylovora* and other diseases caused by *Pseudomonas* and *Xanthomonas* species. Oxytetracycline has been isolated from soil actinomycete *Streptomyces rimosus* (21) and is sold with tradename Mycoshield (as calcium salt, from Syngenta) as a foliar spray.

(ii). **Streptomycin**: It was first commercialized for use in crop protection by Meiji Seika Kaisha Ltd. to control the bacterial rots, bacterial canker and other bacterial diseases. It is very effective against *Xanthomonas oryzae*, *X. citri* and *Pseudomonas tabaci* and used in pome fruit, citrus fruits, vegetables, potatoes, cotton and ornamentals. Streptomycin was isolated from a soil actinomycete *Streptomyces griseus* (Krainsky) Waksmann & Henrici. Commercial formulations of streptomycin for agricultural applications include Cuprimicin 17 by Ingenieria Industrial, Paushamycin by Paushak and AAstrepto by Bayer CropScience. The market for streptomycin is small but consistent.

Based on the above information it is very clear that secondary metabolites from actinomycetes and in particular *Streptomyces* group have immensely contributed in development of novel and ecofriendly agrochemicals.

4. CYANOBACTERIA

Cyanobacteria are important members of the planktonic community that serves as a source of novel antifungal and antibacterial agents with pesticidal potential. The potential use of cyanobacterial metabolites in agriculture has been largely neglected, and the research in this area has been limited to *in vitro* screening. The commercial developments of these metabolites have hardly been explored.

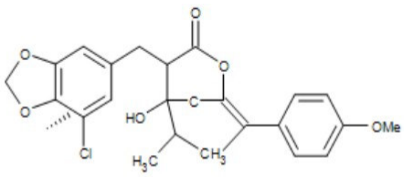
4.1. HERBICIDES

(i). **Cyanobacterin (diaryl-substituted-γ-lactone) (XII):** It is one patented herbicidal compound isolated from cyanobacterium *Scytonema hofmanni* but it has not yet been commercialized (26). Its herbicidal activity is based on the inhibition of Hill reaction as it binds to the QB protein of the photosystem II.

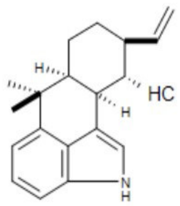
(ii). **Dihydroactiniodiolide:** It was recently isolated from *Phormidium angustissium*, inhibited the activity by interfering in seed germination and seedling growth in *Amaranthus tricolor* L (Chinese amaranth) and *Echinocloa crus-galli* (barnyardgrass) (10).

4.2. INSECTICIDES

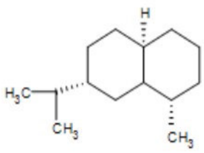
(i). **5(R)-bis-(hydroxymethyl)-3(R), 4(R) -dihydroxy pyrrolidine (DMDP)]:** DMDP, is a pyrrolindine with activity against aquatic insects and crustacean grazers has been isolated from *Cylindrospermum licheniforme* and functions via inhibition of digestive glucosidases in insects and crustaceans (39).



XII Cyanobacterin



XIII 12 epi-Hapalindole J Isonitrile



XIIV Eremophilane

Figure 3. Cyanobacterial compounds as biorational agrochemicals

(ii). **12-epi-Hapalindole J isonitrile, 12-epi-hapalindole C isonitrile and Hapalindole L:** This insecticidal alkaloids was produced by another freshwater cyanobacterium *Fischerella* ATCC43239. The 12-epi-hapalindole J isonitrile **(XIII)** at a concentration of 26 μM inhibits the larval growth of dipteran *Chironomus riparius* (3).

(iii). **Eremophilane (XIV):** It was identified from *Calothrix* sp. PCC7507 which exhibited both insecticidal as well as Anti-crustacean activity. The LC_{50} dose for *Chironomous riparius* and *Thamocephalus platyurus* was 29 and 22 μM, respectively (31).

These trends indicate that cyanobacteria are now attracting crop protection research groups to develop new pesticides.

5. FUNGI

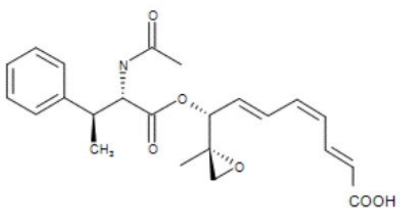
Fungi interact with all forms of life and these interactions could be positive or negative. The interactions between fungi and plants could be pathogenic, mutualistic or endophytic. Plant pathogenic fungi mediate their pathogenesis by virtue of biochemical's which overcome the defence mechanisms of plants and induce wilting, suppression of growth, chlorosis, necrosis and leaf spots. The partial success of fungal biological control agents is attributed to the production of phytotoxins. These have been categorized as (i). Host Specific phytotoxins (HSP's) and (ii). Non Host Specific phytotoxins (NHSP's). HSP's are active towards the plants which are host of the toxin producing fungus and essential determinant for pathogenicity (75) while NHSP's are not primary determinants of pathogenicity and may contribute to the virulence of the fungus. Thus NHSP's exhibit a broader ranges of activity causing symptoms not only to the host plant of the pathogenic fungus but also on other plant species (62).

5.1. HOST SPECIFIC PHYTOTOXINS

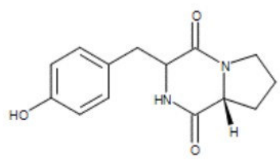
The fungi which produce HSP's are of the genera *Alternaria*, *Cochliobolus*, *Leptosphaeria*, *Venturia*, *Ascochyta* and *Pyrenophora*. Some compounds elaborated by host specific pathogens are so specific that they exhibit toxicity on certain cultivars. The present HSTs are limited to fewer than 20 pathogenic fungi. Among low-molecular-weight HSP's, 7- are from the genus *Alternaria* and 4- from *Cochliobolus* (anamorph *Bipolaris)*. The host selective toxins produced by *Alternaria* species are AK-toxin, AF-toxin, ACT-toxin, AM-Toxin and ACR-toxin.

(i). **AK- toxin (XV):** *Alternaria kikuchiana* is the causative agent of black spot disease of Nijisseiki pears in Japan. The fungus-free culture filtrates *of Alternaria kikuchiana* was toxic to fruits of Nijisseiki, but not to resistant cultivars. This work confirmed the selective toxicity of *A. kikuchiana* culture filtrate suggesting the presence of a host selective phytotoxin which was later identified and named as AK- toxin **(XV)**.

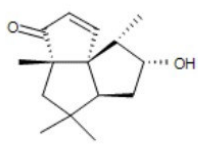
(ii). **AM-toxin:** It is a host specific phytotoxin produced by *Alternaria mali* which induces necrotic spots on leaves of Nijisseiki pears and apples (57). Tangerine pathotype of *Alternaria alternata* causes brown spots in tangerines, mandarins and their hybrids, caused by a host specific phytotoxin called ACT-toxin (49).



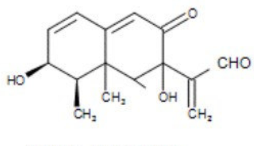
XV AK-toxin



XVI Maculosin



XVII Phomalairdenone



XVIII Bipolaroxin

Figure 4. Host specific phytotoxins produced by fungi

5.1.1. Herbicides:

(i). **AF-toxins:** These are produced by *Alternaria fragariae* infecting Nijisseiki pear and strawberry (70). Maculosin is a cyclic dipeptide, produced by *Alternaria alternata* and is host specific to spotted knapweed (*Cantaeurea maculosa*) (60).

(ii). **Maculosin (XVI):** It is the only host specific phytotoxin for spotted knapweed. Phomalairdenone **(XVII)** is a host-specific sesquiterpene phytotoxin isolated from the *Phoma lingum* (Blackleg fungus).

(iii). **Bipolaroxin (XVIII):** It is isolated from *Bipolaris cyanodontis* (Marignoni) Shoemaker at low concentrations and is host specific i.e. phytotoxic to *Cynodon dactylon* (Bermuda grass), and at 20 times higher concentration is phytotoxic to wild oats, sugarcane and maize.

Host specific phytotoxins have limited or very limited spectrum of hosts, wherein they induce phytotoxic effects and their development and commercialization is very expensive than non-host specific phytotoxins.

5.2 NON-HOST SPECIFIC PHYTOTOXINS

The broader range of phytotoxic activity on different weeds makes non-host specific phytotoxins a suitable candidate for commercial herbicide development in the agrochemical screening programmes. Only few phytotoxic fungal metabolites have been commercialized. Some NHSP's have been discovered and patented for commerical applications.

5.2.1. Herbicides:

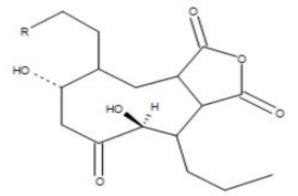
(i). **Cornexistin (XIX):** It is a nonadride phytotoxin from *Paecilomyces variotii* Bainier SANK 21086, exhibiting good herbicidal activity against monocot and dicot weeds and not to maize (71). Cornexistin inhibits aminotransferases (12). LT-toxin from *Lasiodiplodia theobromae* MTCC3068 is an effective herbicide to control *Parthenium hysterophorus*, duckweeds, jimsonweed, prickly sida and *Euphorbia hirta* (72). Phytotoxins which could control the weeds *Lantana camara* and *Parthenium hysterophorus* were isolated from *Alternaria alternata* f.sp. *lantanae* and patented for use as herbicide (73). Their mode of action is not known.

(ii). **Macrocidins** (Macrocidin A (XX) and macrocidin B): These tetramic acids, isolated from the *Phoma macrostoma* infects the *Cirsium arvense* L.(*Canada thistle*) and patented for their herbicidal activity to control weeds (74). These compounds induce bleaching and chlorotic symptoms in broad leaved weeds. These compounds travel through phloem of plants to induce their phytotoxic effects. The macrocidins appear to inhibit the carotenoid synthesis in plants by different mode other than inhibition of HPPD (*p*-hydroxyphenylpyruvate dioxygenase) which produces plastoquinone required for activity phytoene desaturase required for the synthesis of carotenoids (27).

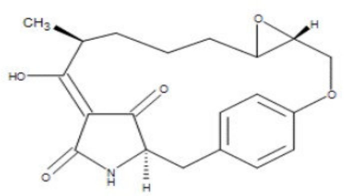
(iii). **Phyllostictine A (XXI):** It is a powerful toxin produced by a mycoherbicide *Phyllosticta cirsii* used for the biological control of *Cirsium arvense*. The toxin has a potential phytotoxic activity and holds a promise for natural herbicide development (78).

(iv). **Zinniol:** It was isolated from *Alternaria cirsinoxia* C-363 E.G. Simmons & K. Mort. and is phytotoxic to *Cirsium arvense* L.and 11 other plant species (4).

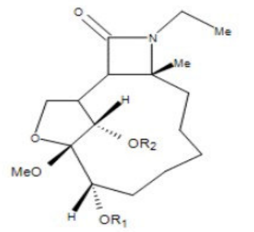
(v). **Cinnacidin (XXII):** It is an NHSP isolated from *Nectria* sp. DA60047, a plant pathogen which causes cankers in many tree species. The symptoms produced by cinnacidin is stunting and chlorosis which spreads throughout the foliar tissues (33). Ascaulitoxin aglycone is a non-protein amino acid glycone of the phytotoxin ascaulitoxin produced by the plant pathogen *Ascochyta caulina*. This toxin acts slowly initially stopping growth but finally resulting in chlorosis and death (18).



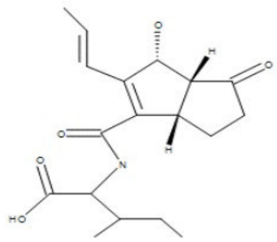
XIX Cornexistin



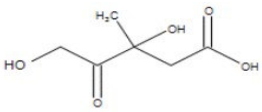
XX Macrocidin



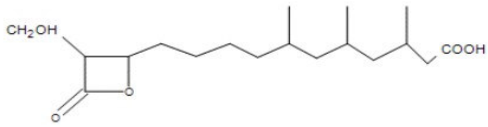
XXI Phyllostictine



XXII Cinnacidin



XXIII Mevalocidin A



XXIV Antibiotic 1233A

Figure 5. Non-Host specific phytotoxins produced by fungi

(vi). **Mevalocidin (XXIII):** It is another new mobile phytotoxin, produced by *Fusarium* DA056446 and *Roselliana* DA092917. It is broad spectrum post emergence herbicide against grasses and broadleaved plants. It has both phloem and xylem mobility (24).

(vii). **Pyrenophorin(8,16-dimethyl-1,9-dioxa-cyclohexadeca-3,11-diene-2,5,10,13-tetraone):** It is fungal phytotoxin from *Pyrenophora avenae* (34), *Stemphylium radicinium* (28) and *Drechslera avenue* (40). It is non-selective toxin that causes chlorophyll retention in leaves known as 'green islands', besides affecting the root growth in graminaceous species (1). The compound exhibits phytotoxic activity by exerting a bleaching effect, accompanied by increased electrolyte leakage, higher superoxide dismutase activity, loss of photosynthetic pigments and decline in total protein. As pyrenophorin exerts its phytotoxic action in both dark and light conditions, its probable mode of action is electron misdirection and generation of ROS (reactive oxygen species)(1).

(viii). **Antibiotic 1233A (XXIV):** It is a phytotoxin also known as hymeglusin or L-659699 produced by several fungal plant pathogens (e.g. *Cephalosporium* sp., *Fusarium* sp., etc). It inhibits 3-hydroxy-3-methylglutaryl coenzyme A(HMG-CoA) synthetase, an intermediate in the mevalonate pathway in plants (17).

(ix). **Tentoxin:** This phytotoxin has potential as herbicide development a cyclic tetrapeptide produced by *Alternaria alternata* causing phytotoxic damage to both moncot and dicot weed species.

5.3 INSECTICIDAL AND NEMATICIDAL AGENTS FROM FUNGI

Fungi also parasitize the insects and nematodes and directly or indirectly play major role in their biological control. Thus secondary metabolites produced by insect and nematode predating fungi may be exploited to develop biorational insecticides and nematicides.

5.3.1 Insecticides

Metarhizium anisopliae and *Beauveria bassiana* are most studied entomopathogenic fungi. The other important members of this class of fungi are *Tolypocladium* spp., *Paecilomyces fumosoroseus, Verticillium lecanii*. These secrete an array of secondary metabolites, restricted to specific genera whereas other is more ubiquitous.

(i). **Destruxin:** Destruxin A **(XXV)** and B were discovered as the first entomopathogenic metabolites produced by *M. anisopliae.* Later many isomers of destruxin were isolated and now there are 28 structurally related destruxins (63). Destruxins A1, A4, A5 and homodestruxin B is produced by the entomopathogenic fungus *Aschersonia* spp.

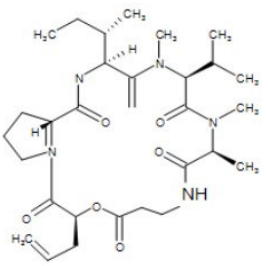
(ii). **Efrapeptins:** These compounds are produced by *Tolypocladium* spp. (41) have miticidal and insecticidal activities against arthropod pests (e.g. spidermites, potato beetles and diamondback moth) (42). Efrapeptins also cause dose dependent anti-feedant and insect growth inhibition (2). The toxin produced by *Beauveria bassiana* is oosporein **(XXVI),** a red dibenzoquinone (20).

(iii). **Oosporein:** Oosporein is major secondary metabolites produced by 3- commercial strains of *Beauveria brongniartii* in submerged cultures and on sterilized barley kernels (63).

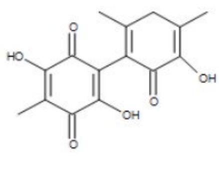
(iv). **Beauvericin:** Beauvericin **(XXVII)** is a hexadepsipeptide produced by entomopathogenic fungi *Beauveria bassiana* and *Paecilomyces* spp. It has two forms (i). beauvericin A and (ii). beauvericin B. Which are very toxic to marine and human cell lines. Till date the secondary metabolites produced by entomopathogenic fungi have not been developed into a biorational insecticide due to their toxic actions on non-target organisms.

Fungal endosymbionants may directly or indirectly affect the insects feeding on fungal host plants. Thus fungal secondary metabolites are helpful to maintain the plant-fungus symbiosis as they alter the feeding behaviour of invertebrates, thereby reducing the invertebrate growth and protecting the host plants from herbivory (43,54).

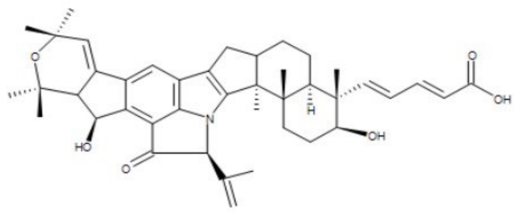
(v). **Nodulisporic acid (XXVIII):** It is produced by the endophytic fungus *Nodulisporium* sp. from the Hawaiian plant *Bontia daphnoides* (56) and has insecticidal activity against fleas by modulating a specific glutamate gated ion channel.



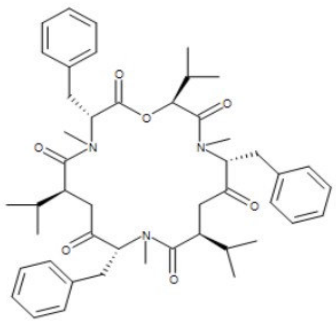
XXV Destruxin A



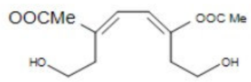
XXVI Oosporein



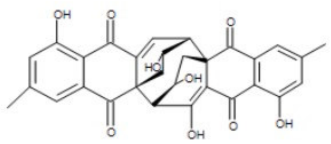
XXVIII Nodulisporic Acid



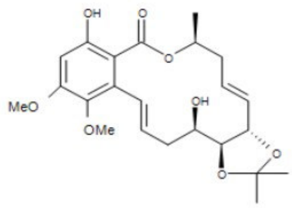
XXVII Beauvericin



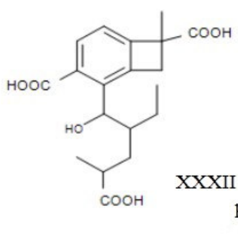
XXIX Citrantifidiene



XXX Rugulosin



XXXI Caryospomycin



XXXII 4-(4'-carboxy-2'-ethyl-hydroxypentyl)-5, 6,-dihydro-6-methylcyclobuta[b]-pyridine-3, 6-dicarboxylic acid

Figure 6. Insecticidal and nematicidal secondary metabolites produced by fungi

(vi). **Rugulosin (XXIX):** Rugulosin is produced by the fungal endophyte *Phialocephala scopiformis* which inhabits white spruce needles. The toxin possesses anti-feedant activity against spruce budworm (*Choristoneurea fumifera*) (53,54).

Non-endophytic fungi also produce insecticidal moieties such as 4-(*N*-methyl-*N*-phenylamine)-butan-2-one, citrantifidiene and citrantifidiol.

(vii). **Citrantifidiene:** Citrantifidiene **(XXX)** and citrantifidiol have been isolated from soil-dwelling isolate of *Trichoderma citrinoviridae* and possess anti-feedant activity against aphids *Schizaphis graminum* (Rodani).

(viii). **4-(*N*-methyl-*N*-phenylamine)-butan-2-one:** It was isolated from *Aspergillus gorakhpurensis* and exhibited a marked larvicidal activity (LD_{50} = 330.69 µg/ml) in *Sodoptera litura* (8).

5.3.2 Nematicides

Fungi also produce many nematicidal compounds, which may be developed as commercial nematicides.

(i). **Omphalotin A:** *Omphalotus olearicus* produces a cyclic dodecapeptide, omphalotin A which has higher efficacy than known nematicides such as the ivermectin in potency as well as in selectivity (50, 51).

(ii). **Caryospomycin:** *Caryospora callicarpa* YMF1.01026 also produces caryospomycin A **(XXXI)**, B and C with potential nematicidal activity to pinewood nematode *Bursaphelenchus xylophilus* (16).

(iii). **Dicarboxylic acid:** Similarly a *Paecilomyces* sp. YMF 1.01761 produces a novel nematicidal compound 4-(4'-carboxy-2'-ethyl-hydroxypentyl)-5,6,-dihydro-6-methylcyclobuta[b]-pyridine-3, 6-dicarboxylic acid **(XXXII).** The LD_{50} values of the compound after 24 h against *P. redivivus* was 50.86 mg/L, against *Meloidogyne incognita* 47.1 mg/L and *B. xylophilus* 167.7 mg/L (46).

5.4 Fungal metabolites as biofungicides

Fungicides are compounds which inhibits the growth of pathogenic fungi in plants and animals. The fungi *Oudemansiella mucida* and *Strobilurus tenecellus* possessed anti-fungal activity but their bioactive compounds, viz. strobilurins, were too photosensitive for commercial use. However advances in synthetic organic chemistry lead to development of photostable derivatives, which are commercially successful as biofungicides and exhibited remarkable activity against many foliar pathogens from the Ascomycetes, Basidiomycetes, Deuteromycetes, and Oomycetes in cereals, rice, pome fruits, grapevine, vegetables, and turfgrass. Structural modification of strobilurins resulted in the development of three analogues with improved photochemical stability namely, azoxystrobin **(XXXIII)**, kreso-oxim methyl, trefloxystrobin, picoxystrobin **(XXXIV)**. Compared to fungicides already in use, strobilurin analogues have greater efficacy and yield responses for several diseases/crops. Additional yield increases of 5-10% are reported in cereals than traditional fungicides.

Endophytic fungi also produces the natural products which could be used as fungicides as under.

(i). **Cryptocin (XXXV):** It is a tetrameric acid analogue isolated from *Cryptosporiopsis quercina* existing as an endophyte in stem of *Tripterigeum wilfordii* and effective against *Pyricularia oryzae,* the causal agent of rice blast disease (45). The other molecule produced from the same endophytic fungi is cryptocandin **(XXXVI)**; it is active against both human and plant pathogenic fungi (64).

(ii). **Cytochalsins**: Cytochalasin H and Cytochalasin N were isolated from *Phomopsis* sp. existing as an endophyte in *Gossypium hirsutum.* The IC_{50} values of these cytochalasins ranged between 0.1 to 50 μg/ml against *Sclerotinia sclerotium, Fusarium oxysporum, Botrytis cineria, Bipolaris sorokiniana* and *Rhizoctonia cerealis* (22).

(iii). **Colletotric acid (XXXVII):** This is a phenolic compound isolated from *Colletotrichum gloesporoides,* which exists as an endophyte in *Artemisia mongolica* and is effective against *Helminthosporium sativum* exhibiting a minimal inhibitory concentration of 50 μg/ml (79).

(iv). **Rufuslactone:** Rufuslactone is a sesquiterpene lactaranae from fruiting bodies of *Lactarius rufus*, a basidiomycete. It is an isomer of 3,8-oxa-13-hydroxylactar-6-en-5-oic acid γ- lactone from *Lactarius necator*. It inhibits the mycelial growth of plant pathogenic fungi viz., *Alternaria brassicae*, *Fusarium graminearum*, *Botrytis cineria* and *Alternaria alternata*. *Alternaria brassicae* (47).

5.4.1 Volatile Biofungicides

Apart from many fungal secondary metabolites being exploited as fungicides, herbicides, insecticides and nematicides (Table 4). The fungi also produce several volatile organic compounds which could be used as a fumigant for the storage of fruits and vegetables. Volatiles of *Muscodor albus* Worapong, Strobel and Hess WM, an endophyte of *Cinnamomum zeylanicum* possess biofumigant properties attributed to over 20 volatile organic compounds (alcohols, acids, esters, ketones and lipids) (76) and prevents or kills the mycotoxigenic fungi (*Aspergillus carbonasius*, *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus ochraceous*, *Fusarium culmorum* and *Fusarium graminearum*) apart from phytopathogenic fungi (6). *Nodulisporium* sp.CMU-UPE34, an endophyte in *Lagerstroemia loudoni* produces anti-fungal volatile compounds (alcohols, acids, esters and monoterpenes) which controls the green and blue mold decay caused by *Penicillium digitatum* and *Penicillium expansum* (65).

6. ADVANTAGES OF MICROBIAL METABOLITES AS PESTICIDES

Finding newer pesticides with novel site of actions is very important as the resistance to currently used synthetic chemicals have increased dramatically and the market niches for the currently exploited sites are reaching saturation (15). Microbial secondary metabolites often possess novel chemical templates which may be developed into biorational ecofriendly counterparts of the conventional agrochemicals. Further these could offer themselves as tools for envisaging new molecular sites of action not discovered by traditional approach of discovery along with safe chemistry. There are little chances of overlap of sites of action of the microbial natural product and conventional agrochemicals. Some microbial natural products can be produced cost effectively via fermentation route as being done to produce the antibiotics in pharmaceutical industry.

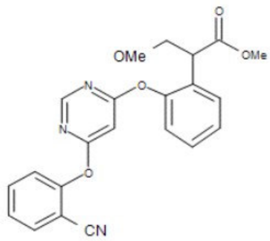
Microbial natural products are (i). Biodegradable and do not leave toxic residues than synthetic organic chemicals used as pesticides, (ii). Application rates are lower than

Table 4. Fungal Secondary metabolites as agrochemicals

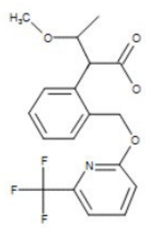
Allelochemical	Source	Bioactivity against organisms				Ref.
		Weeds	Fungi	Nematodes	Insects	
AK- toxin*	*Alternaria kikuchiana*	+	-	-	-	57
AM-toxin*	*Alternaria mali*	+	-	-	-	57
Maculosin*	*Alternaria alternata*	+	-	-	-	60
Cornexistin	*Paecilomyces variotii SANK21086*	+	-	-	-	71
LT-toxin	*Lasiodiplodia theobromae MTCC3608*	+	-	-	-	72
Macrocidin A/B	*Phoma macrostoma*	+	-	-	-	74
Phyllostictine	*Phyllosticta cirsii*	+	-	-	-	78
Cinnacidin	*Nectria sp. DA 60047*	+	-	-	-	33
Mevalocidin	*Fusarium DA056446/ Rosallina DA 092917*	+	-	-	-	24
Pyrenophorin	*Pyrenophora avenae*	+	-	-	-	34
Destruxin A	*Metarrzhium anisopliae*	-	-	-	+	63
Efrapeptins	*Tolypocladium sp.*	-	-	-	+	41
Beauvericin	*Beauveria bassiana*	-	-	-	+	63
Nodulisporic acid	*Nodulisporium species*	-	-	-	+	56
Rugulosin	*Phialocephala scopiformis*	-	-	-	+	53
4(N-methyl-N-phenylamine)-butan-2-one	*Aspergillus gorakhpurensis*	-	-	-	+	8
Omphalotin A	*Omphalotus olearicus*	-	-	+	-	50
Caryspomycin A	*Caryospora callicarpa YMF1.01026*	-	-	+	-	16
4(4'-carboxy-2-ethyl-hydroxypentyl)-5,6,- dihydro-6-methylcyclobuta [b]-pyridine-3,6-dicarboxylic acid	*Paecilomyces sp. YMF1.01761*	-	-	+	-	46
Cryptocin	*Cryptosporiopsis quercina*	-	+	-	-	45,46
Cryptocandin				-	-	
Colletotric acid	*Colletotrichum gloesporioides*	-	+	-	-	79
Cytochalasin N/H	*Phomopsis species*	-	+	-	-	22
Rufuslactone	*Lactarius rufus*	-	+	-	-	47

* Indicate host specific phytotoxins

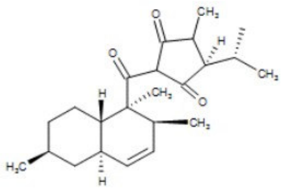
doses of present pesticides. (iii). Contamination of food products, water and soil would be drastically reduced by microbial natural products than present chemical pesticides. The above attributes of microbial natural products seem to befit the complex set of questions put forth by the crop protection research groups and has influenced research in institutions and industries alike to explore the potential of microorganisms.



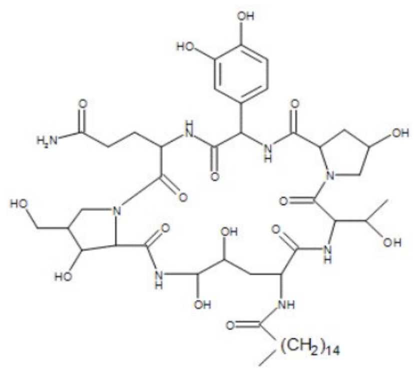
XXXIII Azoxystrobin



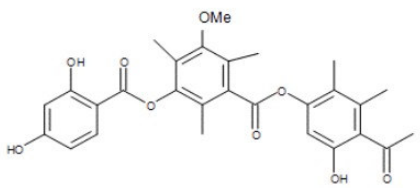
XXXIV Picoxystrobin



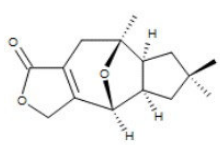
XXXV Cryptocin



XXXVI Cryptocandin



XXXVII Colletotric Acid



XXXVIII Rufuslactone

Figure 7. Fungicidal secondary metabolites produced by fungi

7. CONCLUSIONS

Globally there is an increasing concern regarding the food production to sustain the exponentially increasing human population under a frequently changing environment.

To meet the growing food demand, increased crop production must be achieved without compromising with the quality. The compounds derived from microbes for crop protection may be plausible alternatives for production of quality food without affecting the human health and damaging the environment. Thus it can be concluded that microbial secondary metabolites are alive and offer a great promise in providing new, safe and ecofriendly agrochemicals in the present millennium.

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