2 Secondary Metabolites of *Metarhizium* spp. and *Verticillium* spp. and Their Agricultural Applications

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2.1 Introduction

The practice of cultivation soil and growing of the crops has been one of the major reasons for the adoption of the civilized lifestyle of humans. The practice of agriculture has always been a source of food production as well as a livelihood means. It is estimated that almost one-third of the total crop yield is lost due to the infestation of crop pests, the infection of the pathogens and the competition from the weeds. With the advent of the synthetic pesticides, the loss of the total crop yield was reduced, and the agricultural productivity was increased as it provided protection to the crops against the pests and the diseases. Although the haphazardous use of these synthetic pesticides has led to serious problems such their persistance in environment, residual effects in the food products and development of resistance in pests (Shelton et al. 2002). Over the past few years, there has been an increased concern in the people about the potential adverse effects which are associated with the imperceptive use of the synthetic pesticides, which has, in turn, led to the urge for development of an alternative method for the control of the crop pests (Keswani et al. 2016; Mishra et al. 2015). In this context, microbial secondary metabolites of the reported entomopathogenic fungi are deemed to be employed as one of the finest alternatives.

In general, all microorganisms produce a variety of compound which are structurally related but are found in the different magnitude relatively and are classified as the primary or the secondary metabolites (Singh et al. 2016, 2017). The primary

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metabolites are the microbial products which are made during the log or exponential phase of the growth and whose synthesis is an integral part of the normal growth process on a microbe. These include the end products and the intermediaries of the anabolic metabolism, which are used by the cell as the building blocks for essential macromolecules (such as amino acids, nucleotides) or are converted to coenzymes (such as vitamins). They also include the resultant products from the catabolic metabolism, which leads to the production of energy and utilization of the substrate and thus ultimately to the growth (Sanchez and Demain 2008). The secondary metabolites, on the other hand, are products of the secondary metabolism which are diverse in nature and don't have a role in the basic life processes. They are not involved in either cell metabolism or in the growth of the microorganism. They are produced at the stationary phase of the microbial growth stage and are the source of the therapeutics, insecticides, drugs, flavours and fragrances (Donadio et al. 2002). The concept of the secondary metabolism was first introduced by Kossel in 1891 (Hartmann 1985; Haslam 1986; Seigler 1998; Turner 1971). The application of the secondary metabolites as the insecticide against the crop pests has emerged to be advantageous to other alternatives as they are biodegradable, non-toxic to nontarget organisms and highly selective and have low resistance development in the target pest population (Deepa et al. 2014; Keswani 2015a, b).

A key pest can be described on the basis of economic injury level (EIL), general equilibrium population (GEP) and damage boundary (DB). Pests whose GEP always lies above EIL are persistent, severly damaging and the spray of the insecticides brings their population below EIL. The estimated annual crop loss in India by insect pests is Rs. 29,240 crores (Dhaliwal and Arora 1996).

Most of the studies on the entomophagous fungi are on *Metarhizium anisopliae* and *Beauveria bassiana* and less on equally important species of commercial importance such as *Verticillium lecanii*, *Paecilomyces fumosoroseus*, *Tolypocladium* spp. and *Hirsutella* spp. These fungi produce an array of secondary metabolites, of which some are restricted to specific genera, while others are more ubiquitous (Keswani et al. 2013). These secondary metabolites originate as a derivative from various intermediaries in primary metabolism. In general, most of the secondary metabolites emerge from the five metabolic sources, viz.:

(i) Amino acids
(ii) The shikimic acid pathway for the biosynthesis of aromatic amino acids
(iii) The polyketide biosynthesis pathway from acetyl coenzyme A (CoA)
(iv) The mevalonic acid pathway from acetyl coenzyme A
(v) Polysaccharides and peptido-polysaccharides (Griffin 1994)

This chapter reviews about the different secondary metabolites secreted by the fungi *Metarhizium* spp. and *Verticillium* spp. that act against the crop's insect pests.

2.2 The Hypothesis Suggesting the Role of Secondary Metabolites

There are mainly three hypotheses which suggest the role of the secondary metabolites in organisms which are as follows:

2.2.1 The "Waste Product" Hypothesis

The role of secondary metabolites has been rather uncertain and was initially thought to be just the waste materials. The relatively large number and amount of secondary metabolites which are observed in nature and the concept that these compounds have arisen from the "errors" in the primary metabolisms in plants led to the idea that the secondary metabolic compounds originate and accumulate as "waste products". Although taking into consideration their nonmotility and lack of sophisticated immune system, plants have to develop their own defence system against the pathogens and predators along with the systems to lure the motile organisms, for fertilization and dissemination (Luckner 1972, 1990; Mothes 1976; Seigler 1998).

2.2.2 The Shunt or Overflow Hypothesis

For some, secondary metabolites are envisaged as the shunt metabolic compounds which are produced in a state of unbalanced growth for reducing the abnormal concentration of the normal cellular constituents. The synthesis of enzymes designed to carry out the secondary metabolism allows the primary metabolic enzymes to continue to function until such time as circumstances are propitious for renewed metabolic activity and growth. However, this could be linked to the depletion of nutrients such as phosphorous or nitrogen (Bu'Lock 1980; Haslam 1986).

2.2.3 The Increased Fit Hypothesis

The hypothesis takes into account that many natural products trigger very specific physiological responses in other organisms and in many cases bind to the receptors which have a remarkable complementarity, which means that the natural products may aid in an organism's survival in the absence of an immune system. This fact, in turn, supports the hypothesis that the secondary metabolites increase the fitness of those individuals which possess them and they are favoured in the process of natural selection. The secondary metabolites thus have an important ecological role in the interaction with the environment and act like the communication interface between the plants and its friends and foes in the environment (Harborne 1986; Rosenthal and Janzen, 1979; Swain 1977; Torssell 1997).

Many of those secondary metabolites are fungicidal, bactericidal, repellent or poisonous to insect pests and the herbivores. The flower pigments give attracting colours for insects that help in fertilization or warning colours against the predators. Some of the secondary compounds also perform in signalling pathway as plant hormones (Haslam 1985). In addition to these, many of them are initially meant for defence against herbivores such as insect pests which would soon come up with the metabolic pathways to detoxify and even use these defence compounds.

2.3 The Secondary Metabolites of *Metarhizium* Species

2.3.1 Destruxins

They are a class of cyclic hexapeptides that were originally isolated from the entomophagous fungus, *Metarhizium anisoplae* (Kodaira 1961a, b, 1962; Roberts 1966, 1969). The discussions of the secondary metabolites of *Metarhizium* start and stop with the destruxins. After their first report as insect toxins (Kodaira 1961a, b), several papers and reviews describing their chemistry and biological activities have been published (Hu and Dong 2015; Liu and Tzeng 2012; Pedras et al. 2002).

They are composed of five amino acid residues and a single α-hydroxycarboxylic acid moiety (Suzuki et al. 1970; Suzuki and Tamura 1972; Pais et al. 1981), whose exact nature differentiates the major destruxins into subclasses A to F. The two of the five amino acids are N-methylated amino acids: N-methyl-L-alanine (replaced by L-alanine in protodestruxin) and N-methyl-L-valine (replaced by L-valine in desmethyldestruxin B and protodestruxin). The remaining three amino acids are β-alanine, L-leucine (e.g. in destruxin A and A1 but is replaced by L-valine in destruxin A2) and L-proline (e.g. in destruxin A and A2 but is replaced by L-pipecolic acid in destruxin A1).

The variable structural residue of destruxins is the α-hydroxycarboxylic acid unit. For example, in destruxin A it is 2-hydroxy-4-pentanoic acid; in destruxin B it is 2-hydroxy-4-methylpentanoic acid; in destruxin E it is 2-hydroxy-4,5-methylpentanoic acid; and in destruxin F it is 2,4-dihydroxy pentanoic acid (Wahlman and Davidson 1993). The destruxin analogues obtained from other fungi include destruxins A4 and A5 and homodestruxin B from an entomophagous fungus *Aschersonia* sp. (Krasnoff et al. 1996); roseocardin and roseotoxin B from a plant pathogenic fungus *Trichothecium roseum* (Springer et al. 1984; Tsunoo et al. 1997); bursaphelocides from a *Mycelia sterilia* (Kawazu et al. 1993); pseudodestruxins A and B from a coprophilous fungus *Nigrosalbulum globosum* (Che et al. 2001) and *Beauveria felina* (Lira et al. 2006); and isaridins A and B from an undescribed *Isaria* strain isolated from rat dung (Ravindra et al. 2004; Sabareesh et al. 2007).

Destruxin exhibits an array of amazing biological properties which include insecticidal activity, cytotoxic activity and moderate antibiotic (antituberculotic) activity (Pedras et al. 2002). Apart from these, destruxins have also been shown to possess immunodepressant activity in insect model systems (Vey et al. 1985; Huxham et al. 1989; Cerenius et al. 1990). They cause membrane depolarization by

opening calcium channel leading to the tetanic paralysis in the insects (Samuels et al. 1988). Destruxin E seemed to be the most potent destruxin with the insecticidal activity having repellent and aphicidal properties (Robert and Riba 1989), contact insecticidal activity (Poprawski et al. 1994) and antifeedant properties (Amiri et al. 1999).

2.3.2 Serinocyclins

The serinocyclins were first identified from the conidia of fungus *Metarhizium robertsii* ARSEF 2575, and its structure was elucidated from the isolates obtained from *Metarhizium acridum* (Krasnoff et al. 2007). They are the cyclic heptapeptides which feature many non-proteinogenic amino acids and composed of 1-aminocyclopropane-1-carboxylic acid (ACC) which acylates 4-hydroxyproline followed by the amidation of 1-aminocyclopropane-1-carboxylic acid with L-serine, D-4-hydroxylysine, β-alanine, D-serine and L-serine to form a 22-membered macrocycle. Serinocyclin B has D-lysine in place of D-4-hydroxylysine.

Serinocyclin A showed entomophagous activity as the exposed mosquito larvae to this compound exhibited abnormal swimming as they were unable to control the position of their heads (Krasnoff et al. 2007). The compound is believed to have a neurophysiological effect on the hair tufts which are used as the rudders to adjust the head position while swimming (Brackenbury 1999, 2001). A virtual docking study in 2014 has suggested that the serinocyclin binds to glutathione S-transferase (Sanivada and Challa 2014).

2.3.3 Metachelins

It is a group of coprogen-type hydroxamate siderophores that were isolated first from *Metarhizium robertsii* ARSEF 2575 when it was grown in iron-exploited medium (Krasnoff et al. 2014). The isolated medium included N^{α}-dimethyl coprogen and dimerumic acid which were known earlier to be obtained from *Alternaria longipes*, *Fusarium dimerum* (Jalal et al. 1988), *Alternaria brassicicola* (Oide et al. 2006), *Verticillium dahliae* (Harrington and Neilands, 1982) and *Gliocladium virens* (Jalal et al. 1986), respectively. Apart from these known compounds, four novel siderophores were also reported from *M. robertsii*.

Dimerumic acid is synthesized by the condensation of two molecules of 5-anhydromevalonyl-*N*-5-hydroxyornithine to form a diketopiperazine ring, and further N^{α}-dimethyl coprogen is synthesized by the head-to-tail esterification of the third molecule of 5-anhydromevalonyl-*N*-5-hydroxyornithine to one of the terminal hydroxyl group. One of the four novel siderophores which is also the major component of the mixture, metachelin A, is derived from *N*-dimethyl coprogen molecule after the glycosylation of both terminal hydroxyl groups by D-mannose and N-oxidation of the dimethyl nitrogen.

Metachelin forms the hexadentate chelating complexes with Fe^{+3} and other trivalent metal cations like Al^{+3} and Ga^{+3}. Metachelins and related compounds from *Me. robertsii* showed approximately equal activity to that of the bacterial siderophore, ferrioxamine, in a CAS plate assay (Krasnof et al. 2014).

2.3.4 Ferricrocin

It is an intracellular hexapeptide of the ferrichrome-type siderophore that was produced in its ferrated form by *M. robertsii* 2575 (Jalal et al. 1988). It was first reported in *Aspergillus* spp. (Zähner et al. 1963). It is presumed to receive environmental iron scavenged by extracellular siderophores and to transport it to its target sites in the cell (Wallner et al. 2009). Ferricrocin has the sequence Ser-Ser-Gly-Orn1-Orn2-Orn3-Orn4 where the Orn units are all N^{δ}-acetyl-N^{δ}-hydroxyornithines.

2.3.5 Tyrosine Betaine

It was isolated and characterized from *Metarhizium anisopliae* var. *anisopliae* strain ESALQ 1037 (Carollo et al. 2010). It is a dipeptide molecule having a molecule of betaine that is conjugated with tyrosine whose structure is identified as 2-{[1-carboxy-2-(4-hydroxyphenyl)ethyl] amino}-*N*,*N*,*N*-trimethyl-2-oxoethanammonium (Carollo et al. 2010). It was then also identified in an HPLC screening of the conidial extracts of *Metarhizium acridum* (ARSEF 324, ARSEF 3391 and ARSEF 7486) and *Metarhizium brunneum* (ARSEF 1095, ARSEF 5626 and ARSEF 5749) (Carollo et al. 2010). It is also observed to co-occur with serinocyclins and ferricrocin in extracts of conidia of *Metarhizium guizhouense* (ARSEF 683), *Metarhizium pingshaense* (ARSEF 2106 and ARSEF 5197) and *M. robertsii* (ARSEF 2575 and ARSEF 4123) during mass spectrometric analysis (Donzelli and Krasnoff 2016). The biological activity of this compound has not been reported yet.

2.3.6 Metacytofilin

It is an immunosuppressive compound that was obtained from *Metarhizium* spp. TA2759 which is a two-residue depsipeptide having the structure 3α-hydroxy-6β-methylamino-6α-(−methyl propyl)-3β-phenylmethyl-4H-2,3,5,6-tetrahydro-1,4-oxazine-2,5-dione (Iijima et al. 1992).

2.3.7 Fungerins

Isolated from *Metarhizium* spp. FKI-1079, fungerin, which was initially identified from a *Fusarium* spp. (Singh et al. 2001), along with two novel analogues, namely, hydroxyfungerin A and its regioisomer hydroxyfungerin B, has an imidazole core

(Uchida et al. 2005). The potentiality of the new compound which is unique to the *Metarhizium* strain was 1/12 in acute toxicity assay against brine shrimp (*Artemia*) and was inactive at 10 μg/disk against *Caenorhabditis elegans* or against a panel of microbe which included nine bacteria and five fungi as compared to fungerin (Donzelli and Krasnoff 2016).

2.3.8 Aurovertins

In 2008, three new analogues of aurovertins (F–H) were isolated along with previously described aurovertin D from *M. anisopliae* HF260 (Azumi et al. 2008). Aurovertins were isolated first from *Calcarisporium arbuscula* with the structural elucidation first for aurovertin B (Mulheirn et al. 1974). They are known to inhibit the mitochondrial, bacterial and chloroplast ATPases (F1) and so are used for probing these critical enzymes (Donzelli and Krasnoff 2016).

2.3.9 Metacridamides

The two compounds, metacridamides A and B, were isolated from spores of *Metarhizium acridum* ARSEF 3341 composed of 19-membered macrocyclic lactones (Krasnoff et al. 2012). They neither showed the insecticidal activity nor the antimicrobial activity (Donzelli and Krasnoff 2016).

2.3.10 JBIRs

The compounds JBIR-19 and JBIR-20 were isolated from *Metarhizium* spp. fE61 having two 24-membered macrolides differing from each other by one hydroxyl substitution (Kozone et al. 2009). JBIR-19 showed weak antimicrobial activity against *Saccharomyces cerevisiae* at MICs of 200 μM, but JBIR-20 did not show any antimicrobial activity at this concentration, although both of them induced cell elongation of the same at the concentrations of 3.1 μM and 13 μM, respectively (Kozone et al. 2009).

2.3.11 Helvolic Acid

Helvolic acid was isolated from *M. anisopliae*, and its 6-deacetyl analogue, helvolinic acid, was isolated from *M. anisopliae*, *Metarhizium brunneum* and eight other fungi (Turner and Aldridge 1983). It was originally isolated as an antibacterial "fumigacin", from *Aspergillus fumigatus* and *Aspergillus clavatus*, but was not structurally elucidated (Waksman et al. 1943). The full structure was finally solved in 1970 as a fusidane similar to fusidic acid which is built on the skeleton of cyclopentanoperhydrophenanthrene (Iwasaki et al. 1970; Okuda et al. 1964, 1967).

Helvolic acid along with its 1,2-hydro analogue isolated from *M. anisopliae* strain HF293 was shown to have antibacterial activity against *Staphylococcus aureus* (Lee et al. 2008).

2.3.12 Metarhizins

Metarhizins A and B are the two functionalized diterpenes which are produced by *Metarhizium flavoviride* and are similar to viridoxins (Donzelli and Krasnoff 2016). Metarhizin A has (2R, 3S)-2-hydroxy-3-methylpentanoate at C3 as in viridoxin A, but metarhizin B has (R)-2-hydroxy-3-methylbutanoic acid (deaminated Val) (Kikuchi et al. 2009).

2.3.13 Ovalicins

The type of compound of this group, ovalicin, was isolated from *Pseudeurotium ovalis* (Sigg and Weber 1968). Its hydroxylated analogue, Mer-f3 or 12-hydroxy-ovalicin, was obtained from *Metarhizium* spp. f3 (Kuboki et al. 1999). The ovalicins are monocyclic sesquiterpenoids having highly oxygenated cyclohexane ring and two epoxide groups (Donzelli and Krasnoff 2016). 12-Hydroxy-ovalicin showed immunosuppressive activity in a mixed lymphocyte culture reaction assay and leukaemia cells of L-1210 mouse (Kuboki et al. 1999). It has also shown potent cytotoxicity against four human cancer cell lines and human umbilical vein endothelial cells (Donzelli and Krasnoff 2016).

2.3.14 Taxanes

The overwhelmingly effective chemotherapeutic to cancer, placitaxel, and the other related taxanes were isolated originally from various species of yew trees' bark. Subsequently, placitaxel was reported from an endophyte, *Taxomyces andreanae*, living on Pacific yew (*Taxus brevifolia*) (Stierle et al. 1993). Among the more than 200 reported placitaxel-producing endophytic fungi, the highest yield is obtained from *M. anisopliae* (H-27 Accession FJ375161) (Donzelli and Krasnoff 2016). A controversy attached to the compound is that whether it is indeed a product of fungi at all (Heinig et al. 2013) and, if so, whether it is the result of a fungal version of the accepted plant pathway (Croteau et al. 2006).

2.3.15 Cytochalasins

These molecules are the subset of the "cytochalasans" which were thoroughly reviewed by Scherlach et al. (2010). The first cytochalasans which were described in 1966 are cytochalasins A and B that were obtained from *Phoma* strains S 298

(Rothweiler and Tamm 1966) and *Helminthosporium dematioideum* (Aldridge et al. 1967), respectively, and later cytochalasins C and D were isolated from the cultures of *M. anisopliae* (Roberts 1981). Cytochalasin D is also known to additionally occur in the fungi *Zygosporangium mansonii* and *Helminthosporium* species (Zimmermann 2007). In subsequent years many subclasses of compounds have been put together under the cytochalasins which include scoparisins, chaetoglobosins, penochalasins, aspochalsins, phomacins and alachalasins (Scherlach et al. 2010). In 2000, two new cytochalasin analogues were isolated from *M. anisopliae* in a screen for plant growth retardants, viz. diacetyl-cytochalasin C and an unnamed isomer (Fujii et al. 2000).

Cytochalasans constitute a perhydro-isoindolone molecule which is fused typically with a macrocyclic ring which may be a carbocycle, a lactone or a cyclic carbonate. The cytochalasins bear a benzyl group to the hydrogenated isoindolone moiety. The cytochalasins act as the inhibitors of the actin-cofilin interaction (Roberts, 1981; Strasser et al. 2000). When the plasmatocytes of greater wax moth (*Galleria melanoleuca*) were treated with the cytochalasins obtained from *M. anisopliae*, it was found that it caused the inhibition of attachment and also showed morphological alterations to the untreated ones (Vilcinskas et al. 1997a, b). This inhibition indicates the impairment in the plasmatocytes of the greater wax moth to perform the cell movements required for proper functioning of the cytoskeleton. Despite the basic biological activities of the cytochalasins, they are overshadowed by the destruxins in the collective effort of the secondary metabolites against insects.

2.3.16 Swainsonine

The compound was discovered after the observations of neurological symptoms and weight loss in livestock feeding on *Swainsona* spp. (Family Fabaceae), in *Swainsona canescens*, which inhibited lysosomal α-mannosidase (Dorling et al. 1978). The compound was named swainsonine, and its structure was elucidated as indolizidine-1,2,8-triol (Colegate et al. 1979). It was revealed to be isolated first from the fungus *Rhizoctonia leguminicola* and not from the plant after the complete structural elucidation of a compound that was previously obtained from the aforementioned fungus identical to swainsonine (Guengerich et al. 1973). Swainsonine was then subsequently isolated from *M. anisopliae* F-3622 (Hino et al. 1985). It is an indolizidine alkaloid moiety containing a fused piperidine and pyrrolidine ring system. They act as an aphid-feeding deterrent (Dreyer et al. 1985).

2.3.17 Viridoxins

Isolated from *M. flavoviride* (ARSEF 2133), viridoxins A and B are composed of a diterpenoid core with a 6-methoxy-2,3-dimethyl-γ-pyrone moiety that is attached to the 19th carbon and with (2R,3S)-2-hydroxy-3-methyl pentanoate and

(R)-2-hydroxy-4-methyl pentanoate, respectively, at the third carbon (Gupta et al. 1993). They have shown insecticidal activity against the Colorado potato beetle (*Leptinotarsa decemlineata*) as leaf contamination (Gupta et al. 1993).

2.3.18 *N*-(Methyl-3-Oxodec-6-Enoyl)-2-Pyrroline and *N*-(Methyl-3-Oxodecanoyl)-2-Pyrroline

These are the two substituted pyrrolines that were reported from *Metarhizium flavoviride* HF698 as a weak plant pathogenic oomycete inhibitor (Putri et al. 2014). They were previously reported from *Penicillium brevicompactum* as the juvenile hormone inhibitors and also showed insecticidal activity against *Oncopeltus fasciatus* (Cantin Sanz et al. 1999; Moya et al. 1998) (Table 2.1).

2.4 The Secondary Metabolites of *Verticillium* Species

2.4.1 Bassianolide

It is a toxic metabolite which is obtained from *Beauveria bassiana* and *Verticillium lecanii* (Suzuki et al. 1977), and it was originally isolated from both fungi which were entomophagous on the cadavers of *Bombyx mori* pupae (Murakoshi et al. 1978). The bassianolide is an octadepsipeptide with a 24-membered macrolactone ring which is formed as the cyclic tetrameric ester of the dipeptidol monomer D-hydroxyisovaleric acid-N-methylleucine (Xu et al. 2008). The insecticidal activity of bassianolide was shown by Suzuki et al. in 1977, and it also inhibits acetylcholine-induced smooth muscle contraction (Nakajyo et al. 1983). They are proven to induce atony to the *Helicoverpa* (*Heliothis*) *zea* larvae (Champlin and Grula 1979).

2.4.2 Cyclosporines

They are also called as cyclosporines and were discovered in the 1970s obtained from *Trichoderma polysporum* and *Cylindrocarpon lucidum* (Borel et al. 1977; Dreyfuss et al. 1976). They are a series of cyclo-undecapeptide that were also reported to be produced by the *Verticillium* species by Jegorov and Weiser in 1990. They have insecticidal activities and were reported effective against larvae of mosquito (Matha et al. 1988; Podsiadlowski et al. 1998). Apart from that, cyclosporin A has the immunosuppressive effect on insect humoral immune response (Fiolka 2008) and cellular immune response (Vilcinskas et al. 1999).

Table 2.1 Secondary metabolites from *Metarhizium* spp.

Secondary metabolites class	Metabolite name	Occurrence	References
Peptides	Destruxins	*Metarhizium*	Kodaira (1961a, b)
	Serinocyclins	*Metarhizium robertsii* ARSEF 2575	Krasnoff et al. (2007)
	Metahelins	*M. robertsii* ARSEF 2575	Krasnoff et al. (2014)
	Ferricrocin	*M. robertsii*	Jalal et al. (1988)
Dipeptides and didepsipeptides	Tyrosine betaine	*Metarhizium brunneum* ARSEF 1095	Carollo et al. (2010)
	Metacytofilin	*Metarhizium* sp. TA2759	Iijima et al. (1992)
Amino acid derivatives	Swainsonine	*Swainsona canescens*	Dorling et al. (1978)
	Fungerins	*Metarhizium* sp.	Uchida et al. (2005)
Polyketides	Aurovertins	*M. ansiopliae*	Azumi et al. (2008)
Polyketide/peptide hybrids	Cytochalasins	*M. anisopliae*	Scherlach et al. (2010)
	NG-391 and NG-393	*M. robertsii* ARSEF 2575	Krasnoff et al. (2006)
	Metacridamides	*M. acridum* ARSEF 3341	Krasnoff et al. (2012)
Other polyketide hybrids	JBIR-19 and JBIR-20	*M. anisopliae* var. anisopliae	(Kozone et al. (2009)
Terpenoids	Helvolic acid and related compounds	*M. anisopliae*	Espada and Dreyfuss (1997)
	Viridoxins	*Metarhizium flavoviride* (ARSEF 2133)	Gupta et al. (1993)
	Metarhizins	*M. flavoviride*	Kikuchi et al. (2009)
	Ovalicins	*Metarhizium* sp.	Kuboki et al. (1999)
	Taxol	*M. anisopliae* (H-27 accession FJ375161)	Gu et al. (2015)

2.4.3 Enniatins

They were first discovered in the 1940s (Gäumann et al. 1947). The analogues of enniatin are produced by various species of fungi including *Verticillium* (Herrmann et al. 1996; Supothina et al. 2004). Enniatin molecule is an N-methylated cyclohexadepsipeptides which comprise of three units each of N-methylated branched-chain L-amino acid and D-2-hydroxy acid that are arranged in an alternate fashion (Firakova et al. 2007). They are reported to inhibit ABC transporters (Hiraga et al.

2005), act as ionophores (Levy et al. 1995; Doebler 2000) and suppress acyl-CoA: cholesterol acyltransferase (Tomoda et al. 1992). They have the insecticidal properties (Monma et al. 2006) and are shown to act against the larvae of spruce budworm (*Choristoneura fumiferana*) (Strongman et al. 1988), *Galleria mellonella* (Mule et al. 1992) and adult of the blowfly (*Calliphora erythrocephala*) (Grove and Pople 1980).

2.4.4 Dipicolinic Acid

It is chemically known as pyridine-2,6-dicarboxylic acid. It is the metabolic product of several entomophagous fungi including *Verticillium* spp. (Shima, 1955). Dipicolinic acid was shown to have the insecticidal properties against blowfly (*Calliphora erythrocephala*) (Claydon and Grove 1982).

2.4.5 Verticilides

It was first isolated from the fungus *Verticillium* spp. FK-1033 (Omura et al. 2004). The compound is composed of a 24-membered ring cyclic depsipeptide containing a sequence of cyclo-[(2R)-2-hydroxyheptanonyl-*N*-methyl-L-alanyl] (Omura et al. 2004; Monma et al. 2006). The verticilides are shown to inhibit the ryanodine binding to the ryanodine receptors in cockroach and mouse (Monma et al. 2006; Shiomi et al. 2010).

2.4.6 Enalin

An analogue of enalin A, a coumaranone from the mangrove fungus *Verrucukina enalia* (Lin et al. 2002); 2,6-dihydroxy-2-methyl-7-(prop-1*E*-enyl)-1-benzofuran-3(2*H*)-one was obtained as one of the three compounds from *Verticillium* spp. isolated from the roots of wild *Rehmannia glutinosa* (You et al. 2009). Enalin A is widely distributed from microorganisms to higher plants and is known to have antimicrobial, antifungal, phytotoxic (Furumoto et al. 1997) and antidiabetic (Manickam et al. 1997) activities. The analogue of enalin A obtained from *Verticillium* spp. exhibited antibiotic activity against *Septoria* spp. and *Fusarium* spp. and also inhibited the growth of itself to some extent (You et al. 2009).

2.4.7 Massariphenone

It was originally reported from the marine-derived fungus *Massarina* spp. (Abdel-Wahab et al. 2007). Massariphenone was obtained as one of the three compounds from *Verticillium* spp. isolated from the roots of wild *Rehmannia glutinosa* (You et al. 2009). The chemical formula of the compound was as $C_{10}H_{12}O_3$ by a high-resolution mass spectrometric data, and NMR spectrum of the compound

showed signals of a 1,2,4-tri-substituted benzene ring, an aryl methyl group and an OCHCH3 unit (Abdel-Wahab et al. 2007). It has slight antibiotic activity as it inhibited the growth of *Septoria* spp. and *Fusarium* spp. only slightly (You et al. 2009).

2.4.8 Ergosterol Peroxide

It is reported from a wide range of fungal species and was first obtained from *Cordyceps sinensis* as an antitumor sterol (Bok et al. 1999). It is chemically 5α,8α-epidioxy-24(*R*)-methylcholesta-6,22-dien-3β-ol and was also obtained as one of the three compounds from *Verticillium* spp. isolated from the roots of wild *Rehmannia glutinosa* along with massariphenone and analogue of enalin A. It significantly inhibited biomass accumulation of *Septoria* spp., *Fusarium* spp. and *Rhizoctonia* spp. at a low concentration of 0.97 μg/ml in liquid culture (You et al. 2009).

2.4.9 Radicicol (Monorden)

Radicicol was isolated from *Verticillium chlamydosporium* (= *Pochonia chlamydosporia*) in search for the nematicidal mechanisms from nematophagous fungi (Khambay et al. 2000). It was originally found as an antifungal compound by Delmotte and Delmotte-Plaquee in 1953. Monorden E and an analogue of radicicol, monorden analogue-1, were purified from the fungus *Pochonia chlamydosporia* var. *chlamydosporia* strain TF-0480 (Shinonaga et al. 2009a, b). Radicicol and monorden E were originally obtained from a mycoparasite *Humicola* spp. FO-2942 that produced amidepsines, diacylglycerol acyltransferase inhibitors (Niu 2017). They have antifungal activity only against *Aspergillus niger* (Arai et al. 2003; Yamamoto et al. 2003). Radicicol does not have any nematicidal activity against root-knot nematode *Meloidogyne incognita* (Niu 2017), although it possess antiviral activity against herpes simplex virus (Hellwig et al. 2003).

2.4.10 Pochonins

Pochonins were all isolated first from the strains of *V.chlamydosporium* (= *P. chlamydosporia*) (Hellwig et al. 2003; Shinonaga et al. 2009a, b). Pochonins A–F were isolated from *Pochonia chlamydosporia* var. *catenula* strain P 0297 (Hellwig et al. 2003), and pochonins G–P along with pochonins B, D, E and F were isolated and characterized from *P. chlamydosporia* strain TF-0480 (Shinonaga et al. 2009a, b). Except pochonins F and J, all are chlorine-containing resorcylic acid lactones. Pochonins G and H are the first two compounds in the radicicol family to possess a furan ring, and pochonins L–N are the first three analogues of radicicol with an E-configuration of a double bond at C5–C6. Pochonin K is a 14-aldofuranose radicicol derivative, and pochonin I has a single benzene moiety in the macrolide ring (Niu 2017). Pochonins A–F except for pochonin D showed inhibitory action against herpes simplex virus 1 (Hellwig et al. 2003).

2.4.11 Monocillins

Monocillins I–IV along with radicicol were isolated originally from the fungus *Monocillium nordinii*, a mycoparasite of pine-pine gall rust *Endocronartium harknessii* (Ayer et al. 1980; Ayer and Peña-Rodriguez, 1987). Monocillins are the non-chlorine-containing resorcylic acid lactones. Monocillins II–III along with radicicol, pochonin F and a novel monocillin II glycoside were isolated from *P. chlamydosporia* var. *catenulata* strain P 0297 (Hellwig et al. 2003). All the four monocillins were later isolated from *P. chlamydosporia* strain TF-0480 (Shinonaga et al. 2009a, b). Monocillin III is a dechloro analogue of pochonin A showing potent inhibitory activity against herpes simplex virus 1, and monocillin II is same of pochonin D but with no inhibitory activity to the same virus (Hellwig et al. 2003). Monocillin I has antifungal activities against a wide variety of fungi including *Phycomyces blaksleeanus*, *Pythium debaryanum*, *Ceratocystis ulmi* as the cause of Dutch elm disease and *Phellinus pini* pointing towards the nonspecific nature. However, monocillins II–IV don't show the same antifungal activities (Ayer et al. 1980; Aver and Peña-Rodriguez 1987).

2.4.12 Phomalactones

In 2000, the first study on isolation of phomalactone was reported from the fungus *V. chlamydosporium* (= *P. chlamydosporia*) in a bioassay against the root-knot nematode *Meloidogyne incognita* as a nematicidal metabolite (Niu 2017). It was first isolated from the phytopathogenic fungus, *Nigrospora* spp. (Evans et al. 1969), and was later purified from *Phoma minispora* (Yamamoto et al. 1970; Yamano et al. 1971), *Hirsutella thompsonii* var. *synnematosa* (Krasnoff and Gupta 1994), *Paecilomyces cateniobliquus* (= *Isaria cateniobliqua*) YMF1.01799 (Wu et al. 2012) and *Nigrospora sphaerica* (= *Khuskia oryzae*) (Kim et al. 2001). It has shown nematicidal action against *M. incognita*; dose-dependent insecticidal activity against apple maggot flies, *Rhagoletis pomonella*; and mild toxicity to tephritid fruit flies. Apart from that, it has also shown inhibitory actions to spores of *Beauveria bassiana* and *M. anisopliae* (Krasnoff and Gupta 1994). The growth inhibition of a wide range of microorganisms including fungi, bacteria and a protozoan is shown by phomalactone (Niu 2017).

2.4.13 Aurovertins

Aurovertins D, E, F and I were first isolated from the parasitic fungus of root-knot nematode, *P. chlamydosporia* strain YMF 1.00613 (Niu et al. 2009). Aurovertin D is toxic to the free-living nematode, *Panagrellus redivivus* (Niu 2017).

2.4.14 Pseurotin A

It was originally isolated from *Pseudeurotium ovalis* (Bloch et al. 1976) and is a spirocyclic alkaloid containing oxygen and nitrogen atoms. It was reported as the main metabolite from most of the isolates of *P. chlamydosporia* propagated in Q6 medium (Hellwig et al. 2003). It acts as a chitin synthase inhibitor (Wenke et al. 1993) and has a moderate effect on phytopathogenic bacteria *Erwinia carotovora* and *Pseudomonas syringae* (Niu 2017).

2.4.15 Oosporein

It was originally obtained from a fungus *Oospora colorans* as a red-coloured pigment in 1944 (Niu 2017). It was later obtained from *Verticillium psalliotae* (= *Lecanicillium psalliotae*) that was selected as an antagonist against fungus causing late blight of tomato, *P. infestans* (Wainwright et al. 1986). It has a strong inhibitory action especially against *Phytophthora infestans* (Niu 2017).

2.4.16 Pyrenocines

The first pyrenocines to be described are pyrenocines A and B which were isolated as the phytotoxic metabolites of *Pyrenochaeta terrestris* (= *Setophoma terrestris*) causing pink root disease of onions (Sato et al. 1981). They both were isolated from entomophagous pathogen *Verticillium hemipterigenum* BCC 1449 (Nilanonta et al. 2003). They have several biological activities, reportedly showing phytotoxicity, cytotoxicity and antifungal, antibacterial, antimalarial and antitrypanosomal activity (Sparace et al. 1987; Krohn et al. 2008).

2.4.17 Vertinoids

It is a group of compounds that are obtained from *Verticillium intertextum* ATCC 46284 (Trifonov et al. 1983, 1986). The three secondary metabolites, viz. the hexaketide yellow sorbillin, its derivative 2′,3′-dihydrosorbicillin and the dimeric hexaketide yellow bisvertinoquinol, were reported in 1983, and four new dimeric hexaketides were reported in 1986, viz. bisvertinol, dihydrobisvertinol, isodihydrobisvertinol and bisvertinolone. All the compounds are hexaketide-derived secondary metabolites having two additional methyl groups, one at C2 and the other at C4 of the C12 chain (Niu 2017).

2.4.18 Vertinolide

It was obtained as a new tetronic acid derivative from the fungus *Verticillium intertextum* ATCC 46284 (Trifonov et al. 1982). It contains a 4-hydroxy-3,5-dimethyl-2(5H)-furanone-5-yl and an (E,E)-2,4-hexadienon-1-yl substructures with a dimethylene bridge in between.

2.4.19 Lowdenic Acid

It was isolated from non-sporulating cultures of an undescribed fungus *Verticillium* spp. (MYC-406 = NRRL 29280 = CBS 102427) (Angawi et al. 2003). It has shown antifungal activity against *A. flavus* (NRRL 6541), *Candida albicans* ATCC 90029, *Staphylococcus aureus* ATCC 29213 and *Bacillus subtilis* ATCC 6051 (Niu 2017). Lowdenic acid possesses an unusual bicyclic structure containing a furylidene ring which is linked via a C=C double bond to a tetrahydrofurandione ring (Angawi et al. 2003).

2.4.20 Asteltoxins

They have a trienic α-pyrone structure and are related to citreoviridin and aurovertins. Asteltoxin was the first identified compound as a mycotoxin metabolite of the fungus *Aspergillus stellatus* (Kruger et al. 1979). Later on, four asteltoxin-type metabolites along with two new asteltoxins were isolated from the fungus *Pochonia bulbillosa* (=*Metapochonia bulbilosa*) 8-H-28, which was obtained from the fruiting body of *Elaphocordyceps capitata* (= *Tolypocladium capitatum*) (Adachi et al. 2015). Asteltoxin has shown inhibitory action against *Escherichia coli* BF1-ATPase (Satre 1981).

2.4.21 Bigutol

Bigutol along with its derivative methylbigutol was isolated from the mycoparasitic fungus *Verticillium biguttutum* (Morris et al. 1995). They both have prenylated 4-(hydroxymethyl)benzene-1,2-diol moiety in their structure. Bigutol and methylbigutol both inhibit the growth of *Rhizoctonia solani* and other plant pathogenic fungi (Morris et al. 1995).

2.4.22 Ascochlorin

Ascochlorin-type compounds were first isolated from the fungus *Ascochyta viciae* (= *Septoria viciae*) (Tamura et al. 1968), and later on it has been purified from an array of fungus which includes *Fusarium* sp. LL-Z1272 (Ellestad et al.

1969), *Cylindrocladium ilicicola* (= *Calonectria pyrochroa*) MFC-870 (Hayakawa et al. 1971; Minato et al. 1972), *Nectria coccinea* (= *Neonectria coccinea*) (Aldridge et al. 1972), *Colletotrichum nicotianae* (= *Colletotrichum tabacum*) (Kosuge et al. 1973), *Ascochyta viciae* (Sasaki et al. 1974), *Acremonium luzulae* (= *Gliomastix luzulae*) (Cagnoli-Bellavita et al. 1975), *Cephalosporium diospyri* (= *Nalanthamala diospyri*) IFO 6118 (Kawagishi et al. 1984), *Cylindrocarpon lucidum* (= *Thelonectria lucida*) (Singh et al. 1996), a sponge-derived fungus *Acremonium* sp. (Zhang et al. 2009) and a leafhopper pathogenic fungus, *Microcera* sp. BCC 17074 (Isaka et al. 2015). The metabolites are a class of a 2,4-dihydroxy-5-chloro-6-methylbenzaldehyde (or 5-chloroorclaldehyde) having a sesquiterpene side chain at C5. In 1994, a series of ascochlorin-type compounds which included a new ascochlorin, 8′,9′-dehydroascochiorin, and five known ascochlorins were identified from *Verticillium* spp. FO-2787 (Takamatsu et al. 1994). Again in 2004, a new ascochlorin, 8′-hydroxyascochlorin, and a novel ascochlorin glycoside, vertihemipterin A, together with six known ascochlorins, were isolated from the entomophagous fungus *Verticillium hemipterigenum* (= *Torrubiella hemipterigena*) BCC 2370 (Seephonkai et al. 2004). The members of ascochlorin-type compounds are known to exhibit antifungal activity (Bal Tembe et al. 1999), antiviral activity and antitumour activity (Takatsuki et al. 1969) (Table 2.2).

2.5 The Fate of Secondary Metabolites of *Metarhizium* spp. and *Verticillium* spp.

The two fungi are accessible in the market in both solid and liquid formulations containing the spore and the mycelium of the fungus. When these two entomophagous fungi are applied to a crop ecosystem, it comes in direct contact with humans and target insects and to the crop on which it is applied. The indirect interaction of these fungi happens to occur by drifting to soil, water and atmosphere (Hu et al. 2016). Humans are the first ones to come into contact with the cultures of these fungal entomopathogens whether be it the people who are producing the formulation or the people who are applying it on to their field. There are reports of fungal spore allergy caused by the entomopathogenic fungi including *M. anisopliae* to the workers producing it (Zimmermann 2007), although there are no reports of any sort of things because of the secondary metabolites.

When applied to the crop, the two fungi reach to the proximity of the target insect pests. The spores and mycelium of the fungi adhere to the external surface of insect and then start its infection process. The fungi penetrate through the cuticle of the insect, and various metabolites including various enzymes such as cutinases produced by the fungal mycelia or spores are known to aid in this step of the process. After the penetration, the fungi proliferate itself inside the target insect pest body and then carry on with its life cycle and in the process produce various primary and secondary metabolites. After a successful establishment, the fungi are known to produce the secondary metabolites of which many have the insecticidal effect on the

Table 2.2 Secondary metabolites from *Verticillium* spp.

Secondary metabolites class	Metabolites name	Occurrence	References
Aromatic compounds	ES-242-1	*Verticillium* sp. SPC-15898	Toki et al. (1992a, b)
	ES-242-2	*Verticillium* sp. SPC-15898	Toki et al. (1992b)
	ES-242-3	*Verticillium* sp. SPC-15898	Toki et al. (1992b)
	ES-242-4	*Verticillium* sp. SPC-15898	Toki et al. (1992b)
	ES-242-5	*Verticillium* sp. SPC-15898	Toki et al. (1992b)
	ES-242-6	*Verticillium* sp. SPC-15898	Toki et al. (1992b)
	ES-242-7	*Verticillium* sp. SPC-15898	Toki et al. (1992b)
	ES-242-8	*Verticillium* sp. SPC-15898	Toki et al. (1992b)
	Oosporein	*Verticillium psalliotae*	Wainwright et al. (1986)
Vertinoids	Sorbicillin	*V. intertextum* ATCC 46284	Trifonov et al. (1983)
	2,3-Dihydrosorbicillin	*V. intertextum* ATCC 46284	Trifonov et al. (1983)
	Bisvertinoquinol	*V. intertextum* ATCC 46284	Trifonov et al. (1983)
	Bisvertinol	*V. intertextum* ATCC 46284	Trifonov et al. (1986)
	Dihydrobisvertinol	*V. intertextum* ATCC 46284	Trifonov et al. (1986)
	Isodihydrobisvertinol	*V. intertextum* ATCC 46284	Trifonov et al. (1986)
	Bisvertinolone	*V. intertextum* ATCC 46284	Trifonov et al. (1986)
Furanone and pyranone	Vertinolide	*V. intertextum* ATCC 46284	Trifonov et al. (1986)
	Lowdenic acid	*Verticillium* sp. (MYC-406 = NRRL 29280 = CBS 102427)	Angawi et al. (2003)
	Canescin	*Verticillium* sp. (MYC-406 = NRRL 29280 = CBS 102427)	Angawi et al. (2003)
	Pyrenocin A	*V. hemipterigenum* (teleomorph: *T. hemipterigena*) BCC 1449	Nilanonta et al. (2003)

(continued)

Table 2.2 (continued)

Secondary metabolites class	Metabolites name	Occurrence	References
	Pyrenocin B	*V. hemipterigenum* (teleomorph: *T. hemipterigena*) BCC 1449	Nilanonta et al. (2003)
Phenol-terpenoid hybrids	*Bigutol*	*V. biguttatum*	Morris et al. (1995)
	Methylbigutol	*V. biguttatum*	Morris et al. (1995)
	*LL-Z1272*β	*Verticillium* sp. FO-2787	Takamatsu et al. (1994)
	8′,9′-Dehydroascochlorin	*Verticillium* sp. FO-2787	Takamatsu et al. (1994)
	*Ascochlorin/LL-Z1272*γ	*Verticillium* sp. FO-2787	Takamatsu et al. (1994)
	8′-Acetoxyascochlorin/ LL-Z1272	*Verticillium* sp. FO-2787	Takamatsu et al. (1994)
	8′-Hydroxyascochlorin	*V. hemipterigenum* BCC 2370	Seephonkai et al. (2004)
	Vertihemipterin A	*V. hemipterigenum* BCC 2370	Seephonkai et al. (2004)
	Ascofuranone	*V. hemipterigenum* BCC 2370	Seephonkai et al. (2004)
	Ascofuranol	*V. hemipterigenum* BCC 2370	Seephonkai et al. (2004)
Terpenoids	*β*-Apo-4′-carotenoic acid	*V. agaricinum*	Valadon and Mummery (1977)
	β-Apo-4′-carotenoic acid methyl este	*V. agaricinum*	Valadon and Mummery (1977)
	Dahliane A	*V. dahlia*	Wu et al. (2016)
	Dahliane B	*V. dahliae*	Wu et al. (2016)
	Dahliane C	*V. dahliae*	Wu et al. (2016)
	Dahliane D	*V. dahliae*	Wu et al. (2016)
Nitrogen-containing phenolic compound	*Balanol*	*V. balanoides*	Kulanthaivel et al. (1993)
Cyclodepsipeptides	*Bassianolide*	*V. lecanii* (*Lecanicillium* sp.)	Suzuki et al. (1977)
	Enniatin B	*V. hemipterigenum* BCC 1449	Nilanonta et al. (2003)

(continued)

Table 2.2 (continued)

Secondary metabolites class	Metabolites name	Occurrence	References
	Enniatin B4	*V. hemipterigenum* BCC 1449	Nilanonta et al. (2003)
	Enniatin C	*V. hemipterigenum* BCC 1449	Nilanonta et al. (2003)
	Enniatin G	*V. hemipterigenum* BCC 1449	Nilanonta et al. (2003)
	Enniatin MK1688	*V. hemipterigenum* BCC 1449	Nilanonta et al. (2003)
	Enniatin H	*V. hemipterigenum* BCC 1449	Nilanonta et al. (2003)
	Enniatin I	*V. hemipterigenum* BCC 1449	Nilanonta et al. (2003)
	Enniatin O1	*V. hemipterigenum* BCC 1449	Supothina et al. (2004)
	Enniatin O	*V. hemipterigenum* BCC 1449	Supothina et al. (2004)
	Enniatin O3	*V. hemipterigenum* BCC 1449	Supothina et al. (2004)
Diketopiperazines	*1-Demethylhyalodendrin tetrasulfde*	*V. hemipterigenum* BCC 1449	Nilanonta et al. (2003)
	Vertihemiptellide A	*V. hemipterigenum* BCC 1449	Minato et al. (1973)
	Vertihemiptellide B	*V. hemipterigenum* BCC 1449	Nilanonta et al. (2003)
	Demethylhyalodendrin	*V. hemipterigenum* BCC 1449	Nilanonta et al. (2003)
	Verticillin A	*Verticillium* sp. TM-759	Minato et al. (1973)
	Verticillin B	*Verticillium* sp. TM-759	Minato et al. (1973)
	Verticillin C	*Verticillium* sp. TM-759	Minato et al. (1973)
Polyhydroxylated pyrrolizidin	*Pochonicine*	*P. suchlasporia* var. *suchlasporia* TAMA 87	Usuki et al. (2009)

insect pests that are still living up to this stage of fungal infection cycle. So at last, the fungal establishment and its secondary metabolites along with the cadavers of the target insect pests enter the environment. Till now, estimating the number of the fungal metabolites getting released in the environment is very difficult (Hu et al. 2016), although a few of the research point out that the number of metabolites of the entomopathogenic fungi reaching the environment is scarce. As, for example, destruxins, a secondary metabolite of *Metarhizium* spp., targets insect pests, as the compound decomposes shortly after the death of the host insect pest. The decomposition of destruxin is presumed to be due to the activity of the hydrolytic enzymes in

the cadaver, being independent of host or soil and biota, apparently. Thus, destruxins are restricted essentially to the pathogen and the target host and are unlikely to contaminate the environment or enter the food chain (Skrobek et al. 2008).

The target crop along with the weeds also comes in direct contact with the applied entomopathogenic fungus. As the entomopathogenic species of these two fungi are not phytopathogenic, the fungal mycelial and spores in the suspension just only get deposited on the applied plant's surface but not in those cases where the species of the *Metarhizium* have been shown to possess endophytic characteristics (Mantzoukas et al. 2015).

Next in the line is the indirect interaction that is caused by the drifting of entomopathogenic fungal formulation to the soil, water and atmosphere while applying. The soil is believed to be the reservoir of the microorganisms including the entomopathogenic fungi. The fungus can survive in the soil as a spore or mycelium and/or in form of any dormant or active structures. Drift from the application and the dropping from the target pest cadavers, fungal spores, mycelia and the metabolites can reach the soil system, but there are no such reports of metabolites of fungal entomopathogen being detected in soil (Hu et al. 2016). Although beauvericins have been detected in drainage water after *Fusarium* spp. was inoculated on the wheat plants (Schenzel et al. 2012), there are no reports showing metabolites from these two entomopathogenic fungi reaching the water system, and neither are there reports of the same in the atmosphere (Hu et al. 2016).

2.6 Secondary Metabolites of *Metarhizium* spp. and *Verticillium* spp. as Potent Insecticidal Agents

As discussed earlier, the secondary metabolites are the organic compounds which do not play a direct role in organisms' growth and metabolism (Andersson 2012). Various species of the entomopathogenic *Metarhizium* and *Verticillium* fungi along with the other entomopathogenic fungi have been investigated as a source of a wide range of secondary metabolites which possess bioactivities against a broad range of the insect pests. As a result, diversified metabolites have been reported that display insecticidal properties against insect pests (Khan et al. 2012). Destruxins (A and B) (Kodaira 1961a, b), serinocyclin A (Krasnoff et al. 2007), cytochalasins (Vilcinskas et al. 1997a, b), swainsonine (Dreyer et al. 1985) and viridoxins (Gupta et al. 1993) produced from *Metarhizium* spp. and bassianolides (Champlin and Grula 1979), cyclosporines (Matha et al. 1988; Podsiadlowski et al. 1998), enniatins (Grove and Pople 1980; Strongman et al. 1988; Mule et al. 1992; Monma et al. 2006), dipicolinic acid (Claydon and Grove 1982), verticilides (Monma et al. 2006), phomalactones (Krasnoff and Gupta 1994) and oosporein (Eyal et al. 1994; Wilson 1971) produced from the *Verticillium* spp. are the secondary metabolites that have shown the insecticidal properties apart from the metabolites of other entomopathogenic fungi that have also shown the insecticidal properties. Secondly, there are also certain extracellular enzymes that are produced by *Metarhizium* spp. and *Verticillium* spp. such as chitinase, protease and lipases that also possess certain insecticidal

properties (da Silva et al. 2010). So, proper attention is given on the isolation and purification of such enzymes from their producing entomopathogenic fungal species and their utilization in formulations of biopesticides. Some of these formulations also have been patented by their inventors such as the following: an enzyme preparation composed of at least one protease derived from *Metarhizium*, *Beauveria*, *Verticillium* and *Aschersonia* was formulated and patented (US4987077) (Charnley et al. 1991) and a technology of controlling insect pest prepared with chitinolytic enzymes was patented (US6069299) (Broadway et al. 2000). Similarly, the formulations of the secondary metabolites of *Metarhizium* spp. and *Verticillium* spp. that have shown the insecticidal properties can be engineered and used as target-specific green pesticides.

2.7 Conclusion and Future Perspectives

In this chapter, the secondary metabolites of various *Metarhizium* spp. and *Vetricillium* spp. have been exemplified. Most of these compounds exhibited a profound range of biological activities including antifungal, antibacterial, antitumoral, insecticidal and enzyme-inhibiting abilities. In many cases of the secondary metabolites from these two entomopathogenic fungi, only a superficial research is done except for a few of the metabolites. As the new facets of the secondary metabolites are yet to be explored, there is a wide scope of discovering many new compounds as well as the biological activities of the already discovered compounds. There is also a whole new area of using the secondary metabolites of these two fungi along with the other entomopathogenic fungi as pesticide formulations as only few of them are presently available in the market. Although entomopathogenic fungal formulations are present in the market as suspension of mycelia and spore, there is no any prominent product that uses a secondary metabolite as a pest control. There is a need of a full-fledged research that is focused on finding the novel secondary metabolites, proving the different biological activities of the metabolites, standardization of the effective quantity of the metabolites in their biological activities, finding ways to use the metabolites for human welfare, demarcation of the metabolite use in diverse field of science and technology and adverse effect that may occur due to the metabolites. The major problem of the natural product research is its randomness which is obligated to be rectified. Many new technologies in this particular area are waiting to be revealed and put to use in agricultural and agri-allied sectors and also in other sectors. The specificity in the mode of action of these metabolites makes them eco-friendly and, thus, helps in the sustainable development. With the use of these products, we can maintain the balance in nature while meeting the human demands. It is time we should take keen interest in identification of the natural products of the entomopathogenic fungi as the promising new source of bioactive natural compounds because the time has never been more suitable to do so as now we have all the analytical and the molecular tools at our disposal.

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