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Secondary Metabolites of Entomopathogenic Fungi, Biological Alternative for the Control of Agricultural Pests and Disease: Present and Perspectives

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Abstract

The use of entomopathogenic fungi (EF) in recent years has been highly effective against the different orders of insects considered pests of agricultural importance and their conidia have been commonly applied, but it has been reported that these are sensitive to the environmental conditions. For this reason, biopesticides products have been formulated based on secondary metabolites, recently. These biomolecules participate as biological control agent, such as: cyclic depsipeptides, amino acids, polyketides, polyphenols and terpenoids, affecting their morphology, life cycle and insect behavior. The use of secondary metabolites of entomopathogenic fungi opens the possibility of application in a more efficient way for the control of agricultural pests in a compatible with the environment and human health; therefore, it is important to know, analyzing the type of molecules, their effects, and their different methods of application.

Keywords

Secondary Metabolites, Entomopathogenic Fungi, Biological Control Agent

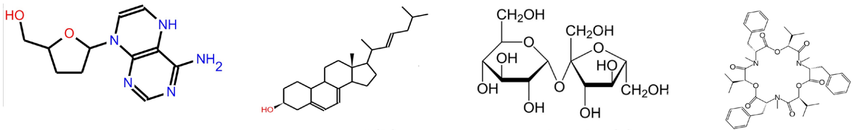
1. Introduction

Entomopathogenic fungi (EF) have shown that they are used efficiently as biological control agents, since they are responsible for causing diseases in insects [1]. Until now, more than 90 genders and 700 species of EF have been described and the isolation of new strains continues, among the most used are: *Metarhizum anisopliae* with 33.9%, *Beauveria bassiana* with 33.9%, *Cordyceps (=Isaria) fumosorosea* with 5.8% and *Beauveria brongniartii* with 4.1% [2] [3]. EF present a life cycle marked by a stage at which conidia are formed as a form of asexual reproduction, in which fungi remain until adhere to the insect's cuticle [4]. According to [5], its pathogenicity mechanism for EF consists of germination, and penetration by mechanical and enzymatic action, mainly by proteases, lipases and chitinases, affecting the host cuticle. Later, [6] mentioned that a germ tube is formed and quickly reaches the hemocele, and the mycelium of the fungus is transformed in blastospores, which evade the insect's immune system, producing toxins, such as secondary metabolites (SM), such as depsipeptides, flavonoids, terpenes, polyketides. SM does not participate directly in the growth of the insect, otherwise, with their survival. SM cause alterations in the epithelial cells of the trachea, disable the mechanisms of the immune system and interrupt the physiological processes of the host, reduce feeding rates and the weight of some insect larvae, damage the nervous system, and they reduce their capacity to defend from the insect [7] [8] [9]. Conidia from EF are generally used in pest control, but EF has a high sensitivity to the variation of climatic conditions such as extreme temperatures and ultraviolet light, and also to their infection time, which is long compared to chemical products [10]. Therefore, there is a potential for formulating new bioinsecticides for the control of agricultural pests from these metabolites [11]. SM can be used together with conidia and thus reduce the time of infection in insects. Therefore, the objective of this review is to analyze the advances in research on the SM produced by EF and the potential they have as biological control agents.

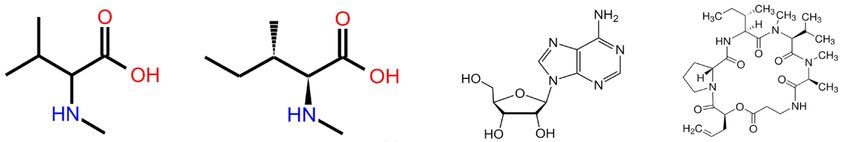
2. Secondary Metabolites of Entomopathogenic Fungi

2.1. Secondary Metabolites

EF produce a wide variety of organic molecules that have no direct function in primary metabolic processes, such as nutrient assimilation, protein, carbohydrate or lipid synthesis, and which are referred to as SM, which are important for the survival and interaction of the organism with its environment [6]. SM serve as: organismis used against other bacteria, fungi, plants, and insects, metal transporters, and symbiosis agents [12]. [13] report that cordycepin (a), ergosterol (b) and polysaccharides (c) (**Figure 1**) are identified as the main metabolites of entomopathogens. On the other hand, other types of compounds have been reported, such as: polyphenolics with functional properties found in the plant and fungal kingdom, such as terpenes, flavonoids and alkaloids [14]. For EF, protein synthesis is essential and ribosomes participate in an important way in this process. However, there are SM called non-ribosomal peptides, which unlike ribosomes, are independent of messenger RNA and among these are polyketides,



Cordycepin (a) Ergosterol (b) Polysaccharides (c) Beauvericin (d)



N-methylvaline (e) N-methylisoleucine (f) Adenosine (g) Destruxins (h)

Figure 1. Cordycepin, ergosterol, polysaccharides and depsipeptides produced by entompathogenic fungi.

non-ribosomal depsipeptides [15] [16] [17]. Several EF species such as *Beauveria spp., Lecanicillium lecanii, C. fumosorosea* and *M. anisopliae* are capable of producing organic acids and some non-peptidic pigments, where these have participated in the infective process against insects considered pests; These compounds have been described as an infectivity factor and it has also been suggested that they may be an element that contributes to the solubilization of the cuticular protein [18].

2.2. Classification of Secondary Metabolites

The template is used to According to their chemical structure, they can be divided into the following groups: cyclic depsipeptides (cyclic tetradepsipeptides and cyclic hexadepsipeptides), peptides (octadepsipeptides, dipeptides and depsipeptides), amino acid derivatives, polyketides, polyphenols, terpenoids as shown in **Figure 1** [19].

a) Cyclic depsipeptides: [20] define cyclic depsipeptides, as a family of cyclic peptide-related compounds, whose ring is mainly composed of amino acid and hydroxy acid residues linked by amide and ester bonds (at least one), resulting in a wide diversity of chemical structures. Beauvericin (d) (**Figure 1**) present in *B. bassiana* and *Fusarium* spp., is a recognized mycotoxin that according to [21] forms a cyclic hexadepsipeptide composed of three molecules of N-methyl phenyl alanine and three molecules of 2-hydroxy isovaleric acid and its synthesis is carried out by a non-ribosomal mechanism specific to fungi using the beauvericin synthetase enzyme complex, which has the ability to increase the permeability of cell membranes to ions causing cell death [22] [23]. These have antibacterial, antifungal, cytotoxic and insecticidal activities, possess low toxicity in humans and do not need special requirements for their use [24]. [25] [26] described that beauvericin belongs to a new group of emerging mycotoxins mainly composed of enniatins (EN) and these are divided into different classes: ENA, ENA1, ENB, ENB1 and BEA, the latter being of most recent interest; the difference between enniatins is due to the amino acid composition and presence of functional groups in different positions, similarly in type A and B EN they are composed of: N-methylvaline (e) or N-methylisoleucine (f) (**Figure 1**) or mixtures of these two amino acids, with a wide range on biological activities. Destruxins (h) (**Figure 1**) are cyclic hexadepsipeptides, isolated mainly from *Metarhizium*, more than 40 types have been reported, of which A, B and E are the most significant in the pathogenesis process and are well known for their insecticidal, fungicidal, and bactericidal activity [27]. Two bioactive compounds have been detected from *C. militaris*; cordycepin (3′-deoxyadenosine), which is an important bioactive compound and has a broad spectrum of biological activity, on the other hand, it has also been reported to produce adenosine (g) (**Figure 1**), which is a nitrogenous base and acts as a cellular nucleoside, which is necessary for the various molecular processes in cells, such as DNA and/or RNA synthesis [28] [29] [30].

b) Alkaloids: Fumosorinone (a) (**Figure 2**) is a 2-pyridone alkaloid isolated from *Cordyceps* (=*Isaria*) *fumosorosea*, it is a potent non-competitive inhibitor of the protein tyrosine phosphatase which is essential in the regulation of cell function in eukaryotes [31] [32]. [33] mentions that in *Cordyceps farinosa*, new yellow pigments, farinosones A (f) and B (g) together with farinosone C (h) (**Figure 2**), a metabolite derived from an early step in the biosynthesis of pyridine alkaloid (b) (**Figure 2**), were isolated. The alkaloid (+) N-deoxy militarinone A was reported by [34] which was extracted from this same fungus, besides three new pyridone alkaloids were isolated from *Paecilomyces militaris* which were: militarinone A, B and D (c, d, e) (**Figure 2**), having a pharmaceutical activity, as well as a biological control agent presenting changes in the host [35] [36].

c) Flavonoids: Flavonoids are MS with broad health-promoting insecticidal use and as biocatalysts, in nature they are mainly found in the form of glycosides [37]. EF are effective in the biotransformation of flavonoids, as demonstrated by [38] obtaining 2′-hydroxy-5′-methyl chalcone 3-O-*β*-D-(4″-O-methyl)-gluco pyranoside from *B. bassiana* and *C. fumosorosea*, also in the latter, flavonones were detected (a) (**Figure 3**) such as 6-methyl 4′-O-*β*-D-(4″-O-methyl)-gluco pyranoside and 2-phenyl-6-methyl chroman 4-O-*β*-D-(4″-O-methyl)-gluco pyranoside.

d) Terpenoids and steroids: Stigmasterol (b) (**Figure 3**), a phytosterol with a side chain containing ten carbon atoms attached to the steroid backbone, was

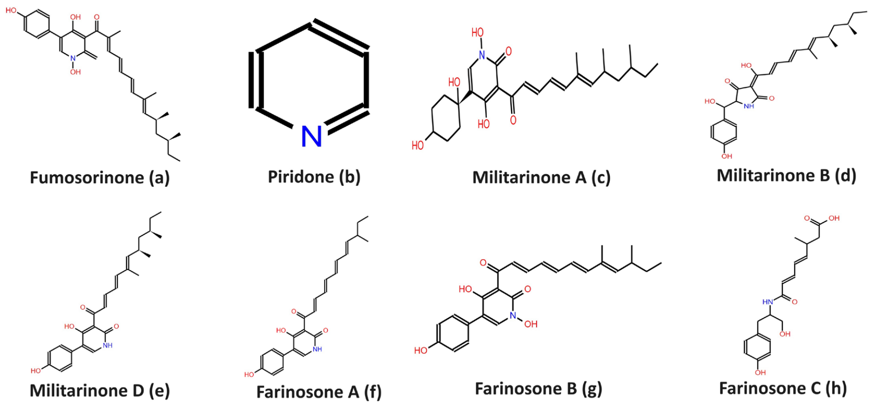

Fumosorinone (a)
Piridone (b)
Militarinone A (c)
Militarinone B (d)
Militarinone D (e)
Farinosone A (f)
Farinosone B (g)
Farinosone C (h)

Figure 2. Alkaloids produced by entomopathogenic fungi.

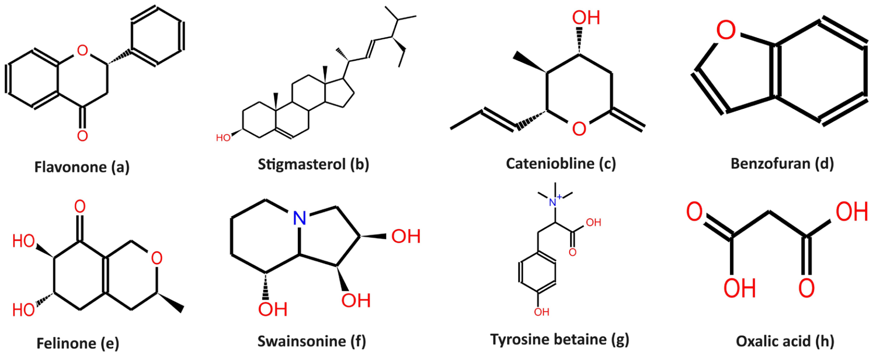

Flavonone (a) Stigmasterol (b) Cateniobline (c) Benzofuran (d)
Felinone (e) Swainsonine (f) Tyrosine betaine (g) Oxalic acid (h)

Figure 3. Flavonoids, terpenoids and organic acids produced by entomopathogenic fungi.

isolated from *Paecilomyces* sp. from marine sponge [39]. On the other hand, [40], report the terpenoid, cateniobline C (c) (**Figure 3**) causing an inhibitory effect on the growth in larvae of *Helicoverpa armigera* [41]. In addition, [32] carried out an analysis of different terpenoids reported in the literature, in which they mention that the benzofuran meroterpernoid was isolated from *Cordyceps annulata*; Benzofurans (d) (**Figure 3**), felinone (e) (**Figure 3**) and chromene meraterpenoids were isolated from *Cordyceps felina*, and they also mention that in *B. bassiana* the following alkaloids were identified: swainsonine (f) and tyrosine betaine (g) (**Figure 3**). Similarly, [42] reported the terpenes: *β*-elemene, *α*-chamigreno and *β*-bisabolene found in *C. fumosorosea*.

e) Organic acids: In *B. bassiana* contains an oxaloacetate hydrolase enzyme in which, through the cytoplasmic way and the mitochondrial pathway, produces oxalic acid (h) (**Figure 3**), which has been reported to have effects involved in the acidification of insect tissues; it also participates in the sequestration of metallic ions such as calcium, manganese, magnesium and iron; participating in the inhibition or reduction of the host defensive system [43] [44] [45]. Another important compound produced by *Cordyceps* spp. and *M. anisopliae* is dipicolinic acid (a) (**Figure 4**) also known as: 2,6-pyridinedicarboxylic acid, where it modifies its pH by acidifying it to allow a greater robustness in the growth of HE conidia as well as, it reduces the sensitivity to ultraviolet rays and finally it has been reported to possess insecticidal properties against the Diptera family. Its mode of action involves cation coordination through the formation of a ligand with three anions that can act as an enzyme inhibitor by subtracting essential ions from metalloenzymes [46] [18] [47] [48].

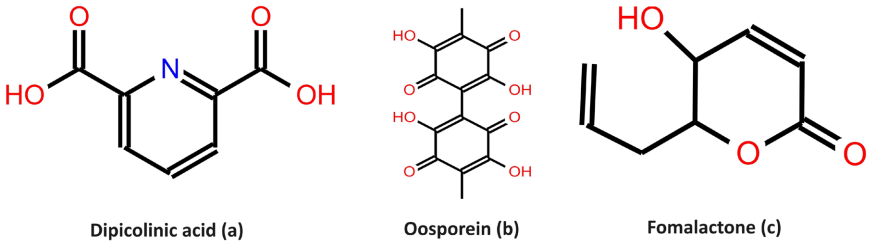

Dipicolinic acid (a) Oosporein (b) Fomalactone (c)

Figure 4. Metabolites produced by entomopathogenic fungi.

f) Non-peptide and polyketide pigments: In the same way, *B. bassiana* is well known to produce a diverse variety of biologically active DMs including non-peptidic and polyketide pigments such as: oosporein (b) (**Figure 4**), bassianin and tenellin [49], the former is a red benzoquinone, which can oxidize proteins and amino acids showing a broad insecticidal, antifeedant and immunosuppressive effect, tested on adults of *Hylobius abietis* (Linnaeus, 1758) and larvae of *Galleria mellonella* (Linnaeus, 1756), on the other hand, bassianin and tenellin affect cuticular membrane ATPases, performing important pathogenesis functions [50] [51].

3. Secondary Metabolites and Its Use as a Biological Control Agent

Secondary metabolites have a broad toxic effect on hosts as it causes severe damage to fatty tissues and organs [52], another reported symptom is the outflow of ions due to the loss of fluids through the peritrophic membrane, causing cellular dehydration through pores, in the same way it has been reported convulsions lack of coordination, altered behavior and paralysis in the host [53], the latter is caused as a consequence of the contraction of insect muscles through muscle depolarization due to the opening of Ca^{2+} channels in the membrane that slows growth [54], also these molecules have effects on nutrient intake in the host presenting a loss of appetite [54] [55]. On the other hand, extracts of phytopathogenic fungi such as: *Fusarium oxysporum* and *Fusarium solani* have insecticidal effect on different orders of insects among them: Hemiptera, Lepidoptera, Coleoptera, Hymenoptera, Blattodea, Diptera, Thysanoptera and Orthoptera , thus finding the greatest effect (96.6%) in the orders: Coleoptera and Diptera [56] [57], Therefore, they have insecticidal effects and can be a form of biological control of insects with a broad spectrum.

3.1. Diptera

Different concentrations of SM belonging *M. anisopliae* have been applied to control larvae of *Anopheles stephensi* (Liston, 1901), *Aedes aegypti* (Linnaeus, 1762) and *Celex quinquefasciatus* (Say, 1823), observing a mortality of 85 to 97% from 24 hours after application, having an effect of these SM against these insects [58]. It has also been showed that extracts of *Lecanicillum attenuatum* isolated from soil with high infective capacity, can be applied against larve of *Aedes albopictus* (Skuse 1895), in the same way it presents negative physiological effects on the same stage of *Plutella xylostella* (Linnaeus, 1758) interfering in its development: such as the inability to withdraw from the head capsule, causing it to not completely remove from its old cuticles [59] called this behavior as insecticidal effect, which is manifested through lack of appetite, inhibition in growth, development and oviposition of the insect. On the other hand, SM of phytopathogenic fungus *F. oxysporum* have also been tested for the control of larvae and pupae of *A. stephensi*, *A. aegypti* and *C. quinquefasciatus* under laboratory conditions; where damage has been detected in the intestinal tissue epithelial cells, adipose tissue and muscles [60]. And phytopathogenic fungus *Ganoderma applantum* has also been used under laboratory conditions for the control of larvae of these same insects, reporting a mortality of 50% after 24 hours of application, observing damage in the epithelial cells of the midgut and damage to muscle tissues in insects [9]. Another method commonly applied for the control of some biopesticides is the use of organic biopreparations such as whey and grain liquor, complemented with *B. bassiana* extract for adults of adults *Drosophila melanogaster* (Fallén, 1823) intensifying its effectiveness, being able to observe 100% mortality two days after application [61]. It can be observed that the synergistic use of these organic biopreparations with the extract of this fungus has a faster effect for the control of this pest.

3.2. Lepidoptera

Cordyceps (*=Isaria*) SM exhibit nematicidal, bactericidal, antiparasitic and insecticidal effects, as is the case of Oosporein (b) (**Figure 4**), which has been used for the control of hemiptera and lepidopteran insects, such as aphids and whiteflies [62]. Furthermore cateniobline (s) (**Figure 3**) and fomalactone (c) (**Figure 4**) in particular, have effects on the growth of *H. armigera* (Hübner, 1805), as demonstrated by [63]. The effect of cordycepin (a) (**Figure 1**), SM produced by *Cordyceps militaris* tested on *Galleria mellonella* (Linnaeus, 1756), has been analyzed and found to cause a significant decrease in the mean survival time of the insect [40]. Similary, crude extracts of *B. bassiana* have been tested on this same insect, producing a 55% larval death, after 96 hours of application [64]. The use of conidia has been the most common for biological control, but the effectiveness of applying extracts of EF has been analyzed, as is the case of the comparison between them by applying them on larvae of two species of *Spodoptera* (Guenée, 1852) under laboratory conditions, where 90% mortality can be obtained with the extract and 30% mortality with conidia for both species after 72 hours of application [15]. And when testing only the SM of this fungus on larvae of *Spodoptera exigua* (Hübner, 1808) and *S. frugiperda* (Walker), a mortality of 26% and 44% respectively was obtained after 7 days of application [65]. The use of fungal extracts is a mixture of several toxins that can produce a potential insecticidal effect together with traditional application of conidia [66].

3.3. Hemiptera

It has been reported that the application of *B. bassiana* extracts has a wide effectiveness for the control of *Bemisia tabaci* (Gennadius, 1889), showing a significant reduction in the population of eggs and nymphs stages [11]. Similarly, extracts of *Lecanicillum lecanii* and *B. bassiana* have also been tested on of *Planococcus citri* (Risso, 1813) nymphs *in vitro*, where it was observed that their extracts produce an insectistatic effect in *P. citri* such as: loss of appetite and poor mobility [6]. On the other hand, MS of *B. bassiana* have been tested of *Aphis gossypii* (Glover, 1877), showing 100% mortality on the third day of application [67]. Similarly the effect that of SM produced by *B. bassiana* on the cellular immune defenses of *Eurygaster integriceps* (Puton, 1881), has been shown to inhibit the phagocytic activity of *E. integriceps* hemocytes and hinder nodule formation and it was also observed that these toxins deactivate several immune mechanisms allowing the fungus to overcome and then kill its host [63]. Dipicolinic acid produced by *C. fumosorosea* from a 6-day supernatant has also been reported to have a toxic effect, an example of which is the mortality observed against third instar nymphs of *B. tabaci* in the first 24 to 48 hours [46] [68].

3.4. Trombidiformes

The combination of plant SM and EF have a wide potential for control of some mites, as demonstrated with the use of 1-chloro octadecane and *Metarhizium anisopliae* extract on *Oligonychus afrasiaticus* (Berlese, 1886), obtaining 100% mortality after 15 days of application, demonstrating their synergy, reducing the mortality time and increasing the insecticidal capacity with this combination [41].

3.5. Prostigmata

The effect of beauvericin against *Tetranychus urticae* (CL Koch, 1836) has been tested with 100% mortality using two different concentrations against motile stages, and it was also found that this toxin was able to inhibit egg hatching up to 69.3 % under laboratory conditions [23].

3.6. Coleoptera

Oxalic acid produced by *B. bassiana* has been found to have insecticidal effects against adults of *Alphitobius diaperinus* (Panzer, 1797), obtaining a mortality of 50.39% [48]. On the other hand, it has been seen that the application of beauvericin (d) in adults of *Hypothenemus hampei* (Ferrari, 1867) negatively affects the insect and blocks its response mechanisms, in the same way it progressively degenerates the tissues, structural changes of the membranes are observed causing changes in the electrical activity of the nerves in the host and this is caused by the increase of oxygen consumption in an attempt of the insect to restore itself [46].

3.7. Nematodes and Others

Beauvericin was shown to have effective insecticidal activity against *Bursaphelenchus xylophilus* (Steiner and Buhrer, 1934), with 46% mortality [25]. Similarly extracts of *B.bassiana* are reported to have a broad effect against *Caenarhabditis* sp. (Dougherty, 1955) obtaining a mortality of 100% at 48 hours of application, it was also tested on *Glycines heterodera* (Ichinohe, 1952), obtaining the same percentage of motality at the same time of application, the same was applied in *Aphelenchoides besseyi* (Christie, 1942), where a mortality of 64.8% was observed in the first 48 hours and finally at the same hours a mortality of 99.7% was found in Meloidogyne incognita [62]. Osporein and beauvericin, besides having an insecticidal effect, also limit the growth of pathogens in plants *in vitro*, so it is inferred that they have fungicidal effects [69]. In the same way, it has been reported that the use of *B. bassiana* extract works as a plant growth factor, for example, increasing the SM production of *Allium schoenoprasum* (Linneo) [70].

4. Conclusions and Perspectives

As previously observed, the highest mortality is obtained when HE extract is applied, and this is due to the fact that this extract contains several toxic molecules in which both enzymes and MS participate, thus increasing toxicity and accelerating mortality. The metabolites of entomopathogenic cannot be synthesized *in vitro* because they are the result of a synthesis by metabolic pathways or by non-ribosomal synthesis peptides [71]. It is important to know how SM are formed and what is their classification, therefore, this review helps us to study the toxins produced by EF, analyzing the different strategies for their application, obtaining highly significant results, having a wide insecticidal capacity both in the larval stage and in insect pupae, as having an insectistatic effect. This effect causes the insects to have changes in their behavior and/or morphology, in which they do not develop normally as if they were 100% healthy, therefore, they become clumsy and thus, susceptible to being prey of other insects, on the other hand, the effect on their behavior is noticeable in their feeding when they stop eating and die. In various studies, it has been shown that the use of conidia and metabolites improves biological control of pests but it depends on the type of pest and its stage [58] [72]. At this time, the production of EF extracts in solid state fermentation maybe it was to be scaled using tray bioreactor and production costs are low because used waste agricultural and marine. Greenhouse evaluation has been carried out using metabolites to control the larvae of *Bradysia impatiens* in *Pinus montezumae* showing that there was no damage to the seedlings [73]. To combat pests in different crops, producers have resorted to the use of organic biopreparations, but by using the extract of an EF in a synergistic way, it increases the infectivity for insects of the order of diptera, so the panorama and a wide possibility of use, is friendly to the environment, as well as to its applicators.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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