The mode of action and mechanism about abamectin

Abamectin has contact toxicity, but its stomach toxicity activity is better than the contact toxicity. After 2-3 days of spraying abamectin, its insecticidal efficacy will be best and the longevity of residues will last about 7-15 days. Although it has no ovicidal action, it can permeate the internal leaves and kill the leaf larvae that hide in the internal leaves and also prevent the newborn larvae from sneaking into the leaves. Besides, it can reduce appetite and egg amount of female adult that contact with the liquid on the blade.

There were a lot of disputes about the mechanism of abamectin pesticide. But until now, more and more research have shown that avermectins is a kind of insecticide that has multiple effects, for example, it can act on the γ-aminobutyric acid (GABA) gated chloride channels, glutamate-gated chloride channel and other chlorine channels.

The effect of abamectin on GABA-gated chloride channels

Early research have shown that abamectin can interdict the muscle excitatory postsynaptic potential (EPSPs) and inhibitory postsynaptic potential (IPSPs) of lobster (Homatus americanus), in addition, it can increase the permeability of chloride ion in the muscle membrane. However, all these effects will be destroyed by GABA antagonist picrotoxin. According to the effect of abamectin insecticide on parasitic nematodes (A. Suum) ventral nerve cord, the synaptic transmission from internuncial neuron to motor nerve cells and the synaptosome peripheral nerve conduction between the neuromuscular are all interdicted. Thus we can infer that this kind of suppression is caused by the open of GABA-gated chloride channels.
With the deepening of the research, people found that abamectin can increase the chloride ion conduction of various vertebrate and invertebrate membranes, which will cause the block of nerve-muscle impulse conduction and the exogenous GABA insensitive of organization receptor. According to the single channel level, when A. suum muscle is dipping into ivermectin, it will activate the compound chlorine ion channel which has sensitive concentration change of chloride ion. By adding GABA into 20nM ivermectin, the opening channel frequency and single channel amplitude will both be weakened. In the determination of chlorine ion flow, the above effects of abamectin mode of action are also proved. By studying the effects of 7 kinds of abamectin analogues on the voltage chloride channels of laboratory rat brain vesicles, Gregory and David indicated that other abamectin analogues all can inhibit the internal flow of brain vesicles GABA chlorine ion flow and stimulate the external flow of insensitive GABA chlorine ion flow except for a kind of octahedral analogue that has no biological activity. It can be seen that avermectins can both inhibit the activation of GABA chlorine ion flow and stimulate the generation of insensitive GABA chlorine ion flow.

On the research of binding site, [3H] abamectin and [3H] ivermectin both have very high affinity with mammals meningeal, of which the Kd value is respectively as 2nM and 22nM. [3H] ivermectin also has high affinity with nematodes (C. Elegans) nerve membranes (Kd value =0.26nM), which may have a close relation to the effects of avermectins on the extensive control nematodes. According to the research on the structure-function relationship of 8 kinds of avermectins analogues, the inhibition combining ability of [3H] ivermectin is closely related with its paralysis ability (r=0.923) on free-living nematodes (C. Elegans). The integrated study on the effects of [3H] ivermectin and [3H] EBOB on housefly nerve membrane shows that avermectins analogues will have very high affinity with housefly head GABA receptor and their specific
binding is about 65 percents, besides, its toxicity is closely related with ligand substitution and it acts on the other site of GABA-gated chloride channels. Based on the ligand binding experiments and electrophysiological experiments on radiolabelling, we can see that the main toxicity mechanism of avermectins is reflected in its inhibiting effect on GABA chlorine ion flow receptor, in which its binding sites are closely matched with the binding sites on EBOB receptor.

**The effects of abamectin on glutamate-gated chloride channel**

Glutamate-gated chloride channel is another kind of channel protein controlling the chloride conduction, which was first discovered in the leg muscle of locust (Schistocetca gregaria) and later cloned its genes from soil nematode (Caenorhabditis elegans). Avermectins analogues can also influence glutamate-gated chloride channel. Under the effect of avermectins analogues, the channels will be activated and the chlorine ion will swarm into the membrane, which will cause the nerve membrane depolarization, block the nerve impulse conduction and lead to muscle paralysis. One of its first evidence shows that ivermectin will induce the membrane conduction irreversible increasing of the locust muscle fibers that have no GABA innervations. The study on the locust muscle fibers which are insensitive with GABA indicates that ivermectin can increase the muscle cell membrane conduction and block the reaction between conduction and ibotenic acid. Ivermectin and water soluble phosphorus analogues can induce the inward chloride electric current of oocytes; while the inward chloride electric current which is sensitive to picrotoxin will be activated by glutamic acid and ibotenic acid instead of GABA or glycine. The electric current caused by glutamic acid and avermectins is similar with the relationship between current and voltage. In addition, the biggest electric current activated by avermectins will reduce the reaction among channels as well as glutamic acid and ibotenic acid. It can be seen that avermectins and glutamic acid can both activate the
same channels which may all become the action sites of avermectins.

The coding expression of glutamate-gated chloride channel will be obtained by adding mRNA of C. elegans into frog oocyte. Experiments indicate that avermectins can activate this channel and the vitro experiments also indicate that the effects of avermectins on C. elegans glutamate-gated chloride channel has good dependency with its activity. This shows that glutamate-gated chloride channel is the action site of avermectins.

The glutamate-gated chloride channels of C. elegans and Drosophila melanogaster have been cloned and they share the similar pharmacology. It is also discovered that α subunit of Dros-Glucl is the action site of avermectins, which can act on the other chlorine ion channels.

Avermectins can also influence on the chloride channels controlled by other ligand (including glycine ligand chloride channel and pressure sensitive chloride channel). By using low concentration of ivermectin (0.1pM) on the lateral contact of crayfish stomach muscles, the composite chloride channel controlled by excited type ligand will be activated, in which the chloride current will be reversibly blocked by the picrotoxin (1mM). Abamectin can inhibit the acetylcholine decomposition of snail nerve cells and induce the irreversible and outward chloride current. In addition, abamectin can non-competitive replace [3H] strychnine which shows that it has certain effects on glycine receptors of mammals.

According to the determination of chloride external flow in rat brain vesicles, we can find the influence of avermectins on pressure sensitive chloride channel. Abamectin can stimulate the chloride external flow in rat vesica, while 100uM of 4’-diisothiocyanostibibene-2,2’-disulfonic acid (DIDS, a kind of pressure sensitive chloride channel blockers) can inhibit 50 percents of its external flow.
By measuring the neurotransmitters released by insect synapses, we can find other effects of abamectin on the chloride channel. Ivermectin can induce insect synapses to release acetyl choline (EC50=20nM) and will reduce the release when the extracellular chloride is increased; while the release will be increased when the extracellular chloride is reduced, which indicates that the depolarization caused by ivermectin can trigger the release of acetylcholine.