**Eco-friendly control management of Alternaria Leaf Spot and**

**Blight Disease through SAR Activation in Plants using Abiotic elicitors**

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1. **Introduction**

Immobile plants have different effective defense mechanisms to recognize and combat as well as resist pathogenic attacks. They are equipped with genetic information which allows them to combat infection by a wide range of pathogenic microorganisms including viruses, bacteria, oomycetes and fungi. These defense responses may be short or long term and are either constitutive or inducible. Thus plants when attacked by pathogens and use several defense mechanisms such as strengthening of tissue at the infection sites, sysnthesis of different types of anti-microbial compounds and synchronous stimulation of defense related genes and their protein products (Agrios, 1997).

As pre-existing defense mechanisms do not protect plants always against plant pathogen attack sufficiently, plants reinforce further protection by developing active defense mechanisms. Several events take place when inducible defenses are activated. The cell wall reinforcement takes place by its components with polyphenolic compounds through cross-linking and by callose (β-1, 3 glucan) deposition. As a result, plants can resist the hydrolytic enzymes of the pathogens (Heath, 2000; Richter and Ronald, 2000). In addition after pathogen recognition, the plants immediately produce phytoalexins and antimicrobial pathogenesis related (PR) proteins (Sticher *et al.*, 1997).

The plant growth regulators, viz. salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) can activate inducible plant defense also (Ton *et al.*, 2002; Beckers & Spoel, 2006). It has been found that to induce resistance in plants there are different signal transduction pathways are required for different pathogens. It has been reported that SA-dependent signal transduction pathways are essential to give resistance to plants from biotrophic pathogens (Glazebrook, 2005), while JA and ET signalling pathways are generally vital for resisting the necrotrophic pathogen attacks. Feys and Parker (2000) further reported that signalling pathways interaction which were salicylic acid (SA) and jasmonic acid (JA)/ethylene (ET) dependent was of primarily mutual antagonism. The plant defense responses against pathogens required to understand the basic plant-pathogen interactions which can be exploited to develop improved disease resistant crops.

It has been reported that in the early 20th Century (Fawcett, 1909), Alternarialeaf spot and blight disease is one of the most widespread fungal diseases of cereals, vegetables, ornamentals etc. belonging to a wide range of families throughout the world (Sharma and Kolte, 1994; Saharan, 1991). A widely diverse pathogen *Alternaria* infects in many crop plants which cause yield losses in a vast amount resulting in reduction in the economic value of the crop plants in conventional production system which are very problematic to manage. One of the common methods of management of Alternariadisease is using of many fungicides which are chemical in nature like difolaton, antrocol, dithane M-45, captan, blitox-50 etc., can satisfactory control plant diseases but they have direct toxic effect on the plant in addition to being are noxious to the ecosystem. Fungicides give short lasting protection to plants with a narrow spectrum of defense which are economically costly too. Management to plant disease control by chemical applications in the form of fertilizers and pesticides is at highly needed for agriculture in growing population in India. However, the continuous applications of these chemicals are resulted in the environmental pollution and may also lead to develop resistance in the target organism. Now a day’s natural farming and organic farming are done in India, it is very important to notice that crop plant disease management also using eco-friendly approaches for maintaining ecosystem, human nutrition and human health through balanced food chain in agro-ecosystems. There is another safest way to control plant diseases is to use resistant or tolerant crop plant varieties. Currently in India, not only there is a serious threat to control by chemical means but there is also a great concern since management of the disease through the development of resistant varieties is not always successful. For this reason, the search for a new economic, eco-friendly and viable alternative means of disease control for Alternaria diseases is highly necessary to stop the hazardous and adverse effects of toxic chemical fungicides and pesticides or their degradation products on environment, beneficial microflora and human health.

Since 1930s, it has been reported that some eco-friendly methods like using biocontrol agents and many plant products to control plant diseases, provides a powerful alternative tool to synthetic toxic chemicals with similar targets as well as induced protection to plants against various pathogens by biotic or abiotic agents. From the late 1950s, there were many evidences which have shown the natural phenomenon of induced resistance and its successful practical application (Kuc, 2001) as an alternative which were less hazardous and economic methods for plant disease control. Disease containment through abiotic elicitors are very eco-friendly noble compounds and now these are becoming an inevitable component in the integrated disease management strategy. After activation of induced resistance in plants, a normally compatible plant- pathogen interaction can be converted into an incompatible one (Mauch-Mani and Slusarenko, 1996; Mauch-Mani *et al*., 1998). For planning long-term sustainable disease management strategies to control Alternarialeaf spot and blight disease, exploration of induction of systemic resistance in plants through the application of biotic or abiotic elicitors can be done.

**2. Occurrence of *Alternaria* and Symptomatology of Alternaria disease**

*Alternaria* species are widespread and can be found in various climates and on a broad range of host plants. They are generally found on agricultural crops such as tomatoes, potatoes, cucumbers, mustard, cabbage, radish and various other fruits and vegetables. They can also affect ornamental plants and trees. Alternaria disease in plants typically manifests as leaf spots, blight, stem cankers, and fruit rot. The symptoms can differ depending on the host and the specific *Alternaria* species involved (Conn and Tewari, 1990). Common symptoms include dark, necrotic lesions on leaves, which may have concentric rings, and cankers or lesions on stems and fruit that often appear dark brown or black.

*Alternaria* infection on different crops is causing world-wide economic loss. among the different diseases caused by the genus *Alternaria.* Among the different diseases caused by the genus *Alternaria*, Alternaria blight and leaf spot diseases are the most dominant disease which causes 32-57 per cent average yield loss (Conn and Tewari, 1990). According toSharma *et al.* (2005), leaf spot on apple was caused by *Alternaria alternata* and firstly the symptoms appeared as reddish brown necrotic spots with 1.3 mm diameter on young apple leaves, later many spots became coalesce to develop larger blotches and also given blighted appearance to the leaves and finally the infected plant became defoliated.

Kamalakannan *et al.* (2008) reported that *Alternaria alternata* (Fr.) Keissler agg was the causal organism of leaf spot diseases on *Aloe vera*. In 2010, Vikas *et al*. has worked on the Alternaria leaf blight in sunflower and reported that the symptoms developed in the form of some characteristic small circular, brown patches on the leaves surfaces of sunflower. At the advanced stage of the disease, these brownish patches expanded in size and later on these patches coalesced to cover the entire leaves surfaces developing blight symptoms. Finally, the blighted leaves got curled and turned to dark black in colour. In 2011, Zhang *et al.* noticed that in early summer the symptoms of *A. alternata* on stems of *Euphorbia lathyris* were characterized by small, brown to black, circular or irregularly shaped spots or/and on shrivelled leaf apices. Later on, in the rainy season it has been found that the spots rapidly grew around the stems or along the leaf blades. According to Shokooh and Shervin (2012), at the initial stage of the of *A. alternata* infection, the symptoms on gerbera leaves were small, scattered, brown spots that gradually became characterized by irregular or round spots and later those spots coalesced to affect large areas of foliage causing defoliation.

Many species of *Alternaria* also can produce toxins that diffuse into host plants as a result a yellow halo or chlorotic halo (Humpherson-Jones, 1992) developed that fades into the healthy parts of host plant tissues which surround the target spot. Dark, sunken spots or lesions are produced on different host plant parts like roots, tubers, stems, and fruits. The pathogens also infect on many weed species, which are inoculum reservoirs (Farr *et al*., 1989; Cucuzza *et al*., 1994; Maringoni, 1997).

1. **The genus *Alternaria***

*Alternaria* has more than 50 species (Ellis, 1971; Anuj Mamgain *et al.*2013). *Alternaria* species are either parasites or saprophytes. Pathogenic *Alternaria* has a very broad range of host. *Alternaria* species can be recognized very easily by their large polymorphous conidial morphology. According to Ellis (1971), conidia are typically ovoid to obclavate, pale brown to brown and formed in chains (catenate) or solitary, often beaked, multicelled and muriform.

1. **Nature of Systemic acquired resistance (SAR):**

Induced resistance in plants against pathogens can be classified into two broad categories viz., (1) systemic acquired resistance (SAR) and (2) induced systemic resistance (ISR). Systemic acquired resistance (SAR) is a mechanism of induced defense which is the ability of plants to give long time protection themselves against wide range of pathogens (Ryals *et al*., 1996; Van Loon, 1997). Over 100 years, SAR has been recognized as a plant response against pathogen infection (Chester, 1933).

After the development of necrotic spots or lesions in host plant, either as a part of the hypersensitive response (HR) or localized acquired resistance (LAR) or as a disease symptoms (Van Loon, 1997), SAR is activated in infected plants via signalling molecule salicylic acid depended process (Hammerschmidt and Becker, 1997; Hammerschmidt, 1999) and is related with the accumulation of pathogenesis-related proteins (PRs) and increased activity of several defense related enzymes, which develop increased level of resistance at the distal, uninfected plant parts against secondary pathogen attack (Kuc, 1995; Ryals *et al*., 1996). Evidence has shown that a decrease in disease symptoms was occurred due to faster and stronger activation of defense responses at the sites of secondary infection which results in (Durrant and Dong, 2004) reflecting the SAR state (Ross, 1961). SAR establishment in plants includes the generation and transport of signals via phloem to the uninfected distal tissues (Guedes *et al.*, 1980; Tuzun and Kuc, 2001) and gives a long-time protection from disease that can last for weeks to month, and sometimes throughout an entire season (Kuc *et al*., 1987).

SAR also can be induced in several plant species by exogenous application of Salicylic acid (SA) (van Loon *et al*., 2006; Pieterse *et al*., 1998; Kessmann *et al*., 1994). The classic form of SAR can be activated biologically by challenging a plant to virulent, avirulent, and non-pathogenic microorganisms as plant defense activators. But, the use of this form of plant inoculation is limited in the agricultural field because of the likelihood of pathogenic microbes spread after application. Although, there are some microorganisms which are commercially available in market as biocontrol agents for use and nearly all are based on a direct antibiotic principle. In addition, exposing a plant to natural or synthetic chemical elicitors such as salicylic acid, 2,6-dichloro-isonicotinic acid (INA) or benzo (1,2,3) thiadiazole-7-carbothioic acid *S*-methyl ester (BTH), β-Aminobutyric acid (BABA) etc., can induce SAR in plants (van Loon, 1998; Laura Mejía-Teniente *et al*., 2010).

1. **SAR Elicitors and Mode of Action of Elicitors:**

The use of SAR for controlling Alternaria infection in plants in an eco-friendly manner is a promising approach. Enhancement of the plant resistance against pathogens, SAR involves inducing a plant's natural defense mechanisms. This method can reduce the need for chemical pesticides, fungicides etc., minimizing the environmental impact. According to research, it is suggested that SAR can effectively suppress Alternaria blight and leaf spot disease by priming the plant's immune system. However, the success of SAR depends on various factors like plant species, timing of induction, and environmental conditions. It's a sustainable strategy worth exploring further, as it aligns with eco-friendly agricultural practices.

The eco-friendly management of Alternaria leaf spot and blight disease through Systemic Acquired Resistance (SAR) and its elicitors presents a comprehensive approach to control plant diseases. SAR involves activating a plant's innate defense responses, enhancing its resistance to pathogens like *Alternaria* sp. Elicitors are compounds that trigger SAR, amplifying the plant's defense response. This SAR inducing strategy minimizes reliance on chemical treatment which is benefiting the environment in a sustainable manner. Research also suggests that SAR, coupled with well-chosen elicitors, can effectively mitigate Alternaria leaf spot and blight. The combined approach promotes sustainable agricultural practices, reducing hazardous negative ecological impacts. However, practical implementation of inducing SAR in plants against pathogens requires consideration of various factors, including plant specificity, elicitor application methods, and local conditions. Overall, the integration of SAR and elicitors holds promise for eco-friendly management of crop plants disease.

The application of abiotic elicitors for eco-friendly management of Alternaria leaf spot and blight is a noteworthy approach. Abiotic elicitors are non-living compounds that can stimulate a plant's defense mechanisms, enhancing resistance against various and detrimental pathogens like *Alternaria* sp. These elicitors can include substances like salicylic acid (SA), 2,6-dichloro-isonicotinic acid (INA) or benzo (1,2,3) thiadiazole-7-carbothioic acid *S*-methyl ester (BTH), β-Aminobutyric acid (BABA) etc., (van Loon, 1998; Laura Mejía-Teniente, 2010). or even nanoparticles, which have shown promising results in inducing systemic acquired resistance. However, the success of this plant disease control management approach depends on factors such as the choice of elicitors, application methods, and specific crop requirements. While abiotic elicitors offer an environmentally friendly alternative, further studies and field trials are essential for optimizing their efficacy in real-world scenarios.

In past, originally the elicitor was used for inducing the synthesis of phytoalexins while at present elicitors are generally applied as compounds which stimulate any type of plant defense (Sinha P.P.and Prasad RK.,1989 Boller T.,1992). Normally, the induction of defense responses may lead to increased level of disease resistance against plant pathogens. SAR elicitors are the products of pathogen origin (exogenous elicitors) and also the compounds which released from infected plants after pathogen infection due to pathogen action (endogenous elicitors) (Ebel J. and Cosio E. G.,1994; Boller T.,1995) .

Elicitors are categorized depending on their origin and molecular structure as physical or chemical, biotic or abiotic and complex or defined. Elicitors can also be classified into two types, “general elicitors and “race specific elicitors. General elicitors are able to activate defense against pathogens both in host plants and non-host plants and in case of race specific elicitors, it induces defense responses only in specific host cultivars which leads to disease resistance. The cultivar specific (gene-for-gene) disease resistance is determined by a complementary pair of genes in a particular pathogen race and a host cultivar. An avirulence gene (avr gene) which is present in a specific race of a pathogen encodes a race specific elicitor and this can elicit disease resistance only in a host plant variety which is carrying the corresponding resistance gene. This has been reported that plant disease is resulted in the absence of either gene product (Hammond-Kosack K. E. and Jones J. D. G.,1996; Cohn J., Sessa G., and Martin G. B., 2001). According to Nürnberger (1999), general elicitors give signals about the presence of potential pathogens to both host and nonhost plants (Nürnberger, T., 1999). Shibuya, N. and Minami, E. (2001) and also reported that the nature of general elicitors is nonspecific which is relative, although some elicitors are only recognizable by a restricted number of plants. At low concentrations, elicitors act as signal molecule in signal transduction mechanism in host plant as signal compounds which provide information for the host plant to trigger primary immune response.

1. **Induction of SAR in plants with abiotic elicitors:**

In a field trial experiment, foliar applications of the synthetic activators like INA or BTH on cotton (*Gossypium hirsutum, G. barbadense*), the disease severity of natural infection with *Alternaria macrospora, Verticillium dahlia* and *Xanthomonas campestris pv. malvacearum* was evaluated (Colson-Hankse *et al*., 2000). Percentage leaf area in cotton plants inoculated with *A macrospora* was significantly (P=0.05) lower following one application of INA or BTH respectively over control and also reported that in the treated plants had significantly (P=0.05) less defoliation. SAR induction following foliar application of INA or BTH significantly reduced susceptibility of the cotton (*Gossypium hirsutum*) plants to *Alternaria* leaf spot, bacterial blight and *Verticillium* wilt.

Some elicitors like antagonistic yeast *Cryptococcus laurentii*, salicylic acid (SA), oxalic acid and calcium chloride induced defense responses and the possible mechanism of induced resistance in harvested pear fruit (*Pyrus pyrifolia* L.cv.Yali) was observed against postharvest disease *Alternaria* rot (Tian *et al*., 2005). The experiment showed that all elicitors increased defense related enzyme activities significantly, such as β-1,3-glucanase, phenylalanine ammonia lyase, peroxidase, and polyphenol oxidase activity and significantly (P=0.05) reduced the disease incidence caused by *A. alternata* in pear fruit. Among all these elicitors, SA treatment was known to be the best abiotic elicitor which induced the defense responses resulting in reduced decay in pear fruit.

Chitra and co-workers (2006) investigated the effect of SA as SAR inducer in groundnut plants to induce resistance against *A. alternata*. The result has shown significant reduction in the leaf blight disease intensity and increased level of the pod yield by foliar application of SA at the concentration of 1 mM under glasshouse conditions. They reported the activities of PAL, chitinase, β-1, 3-glucanase and in phenolic content became changed in groundnut after SA application and *A. alternata* inoculation. It was observed that pre-treatment with SA in plant leaves showed an increased level of phenolic contents after five days challenge inoculation with *A. alternata* in groundnut plants pre-treated with SA. There was a significant increase in chitinase activity in SA-treated pathogen inoculated leaves. Increased activities of chitinase, β-1,3-glucanase were reported in SA treated groundnut leaves. Even peroxidase and polyphenol oxidase activities became induced after foliar applications of SA, upon challenge inoculation with pathogen in groundnut.

Flors and co-workers (2008) conducted an experimental set up in *Arabidopsis thaliana* against the hemibiotrophic pathogen *Pseudomonas syringae* and the necrotrophic pathogen *A. brassicicola* to understand the key role of the callose synthase PMR4 in case of basal resistance and β-Aminobutyric acid-induced resistance (BABA-IR). Treating the host plants with BABA triggered the enhanced levels of PR-1 gene expression and further increased the level of resistance in pmr4-1 mutant. While the enhanced susceptibility to *A. brassicicola* was observed in pmr4-1 plants but failed to show in BABA-IR. The pmr4-1 mutant produced lesser amount of JA upon *A. brassicicola* infection than the wild-type. It was revealed that SA accumulation blockage in pmr4-1 mutants restored basal resistance, but not BABA-IR against *A. brassicicola*. So, it is suggested that the mutant’s increased susceptibility to *A. brassicicola* is caused by SA-mediated suppression of JA, while the lack of BABA-IR is caused by its inability to callose formation. Suppression of ABA accumulation also found in *A. brassicicola* infected plants. Early blight disease caused by *A. solani* in tomato plants (*Lycopersicon esculentum* Mill) was treated with five antioxidants (citric acid, salicylic acid, benzoic acid, ascorbic acid, and sodium citrate) to see the effects on the resistance *in vitro* and *in vivo* study was done by Awadalla, (2008). The results showed that *in vitro* application of all the five antioxidants at the highest concentration (10.0 mM) significantly inhibited the mycelial growth of *A. solani* and the degree of inhibition was directly proportional to the concentration of antioxidants. The applicable concentration ranges of salicylic acid, citric acid and ascorbic acid were found to be more effective to inhibit the mycelial growth than sodium citrate and benzoic acid respectively. During in *vivo* study tomato seeds soaked with all five antioxidants prior to tomato seed sowing showed increased levels of resistance to early blight pathogen, *A. solani*. With varied concentration all five antioxidants markedly reduced the characteristic symptoms of early blight disease on tomato plants and complete inhibition of the disease incidence was at the highest concentrations of each antioxidant tested. Salicylic acid, ascorbic acid, and citric acid were found to be the most effective in controlling the disease and then followed by sodium citrate, and benzoic acid. Increased level of Phytoalexin (tomatine) synthesis was also found greatly in inoculated tomato plants which were antioxidant-treated.

To study the efficacy of seven SAR activators, 2,6-dichloroisonicotinic acid (INA), benzo- thiadiazole S-methyl ester (BTH), β-aminobutyric acid (BABA), K2HPO4, K3PO4, Ca(OH)2 and CaCO3 to induce SAR for the control of *Alternaria* leaf blotch of apple, Sofi *et al*. (2013) applied all these SAR activators 48h before and after *Alternaria mali* spore inoculation on Red Delicious apple cultivar which were two year old grafted seedlings . Evaluation of treatment with the SAR activators along with a conventional synthetic fungicide (penconazole) against most virulent isolate Am-1was studied. the disease intensity was decreased in all the SAR activators treated plants significantly as compared to control (only dist. Water). BABA was noticed to be the most effective with least disease intensity before and after pathogenic inoculation in treated plants which followed by penconazole. Application of SAR activators before pathogen inoculation in plants showed significantly reduction in the disease intensity (12.71%) when compare with SAR application after pathogen inoculation (14.77%). This induction of disease resistance mechanism exploiting natural defense mechanisms of plants could be proposed as a eco-friendly and non-conventional approach for plant protection from various pathogens.

Chavan and Kamble (2013) experimented on application of β-aminobutyric acid (BABA) exogenously on leaves to evaluate disease severity inoculated with *A. brassicae*. As a result disease severity was the reduced in *Brassica carinata.* Changes in defense-related enzymes like phenylalanine ammonia lyase (PAL) and polyphenol oxidase (PPO), isoform analysis of superoxide dismutase (SOD) and peroxidase (POX) were reported to understand the induction of SAR in treated *Brassica carinata* plants. BABA-treated plants have shown a significant level of increased PAL, PPO enzyme activities and total phenolic content in elicitor treated pathogen inoculated plants. While isoform analysis of SOD and POX showed no change in number of isozymes but resulted in a quantitative change in enzyme activities in response to pathogen in treated plants.

Thakur (2014) studied the role of elicitors such as SA and BTH for inducing disease resistance to control *Alternaria* blight in *Brassica juncea* (cv. PBR-91) and *B. napus* (cv. GSC-6) *in vitro*. The effect of elicitors at different concentrations on defense related enzymes *viz.*, peroxidase (PO), phenylalanine ammonia lyase (PAL), superoxide dismutase (SOD); pathogenesis related (PR) proteins *viz*., chitinase, β-1,3-glucanase; phenolics; pigments; ascorbic acid and sugars was investigated in two *Brassica* species. Elicitor treatments enhanced the defense related enzyme activities and phenolic contents as compared to control plants where only water applied. Similarly, contents of total soluble protein, free amino acids, ascorbic acid, total sugars, reducing sugars and photosynthetic pigments were recorded maximum in elicitor treated plants than control, in both the species. Application of all elicitors in the form of combinations of elicitors like BTH (3 ppm) + SA (33 ppm) and BTH (7 ppm) + SA (17 ppm) were found most effective. Disease severity was found to be less in plants which were treated with the combinations of elicitors. Enhanced level of defense related enzymes and phenolic contents reduced the disease severity and increased seed yield. A well-known fact is that elicitors like BTH and SA play a vital role in enhancing the defense mechanism against wide range of pathogen in various plants species. In addition, the study regarding the application of different elicitors in combinations showd markedly effect on inducing the defense response in *B. juncea* (cv. PBR-91) and *B. napus* (cv. GSC-6) against *Alternaria* blight. It has been reported that application of both biotic and abiotic agents induce resistance in plants but chemicals are better as elicitors or inducers, because of it’s formulation and handling and less sensitivity to the environment than biological inducers (Kuc, 1995) as well as these chemical inducers may be better means of applicable possibilities to induce disease resistance because of its easy accessibility and harmless nature. The induced resistance through applying abiotic factors for controlling the plant diseases has been considered to be a great potential approach (Latha *et al.*, 2009). There are certain biochemical changes that occur in plants after the application of resistance inducing agents or elicitors and which play a role as markers for induced systemic resistance (Schönbeck *et al.*, 1980). These biochemical changes include the accumulation of certain defense related enzymes and phenolic compounds (He *et al.*, 2002).

1. **Conclusion**

The pathogen *Alternaria* species are either parasites or saprophyte and noxious pathogen which cause massive destruction in economically important crops and vegetable plants. But the use of advanced knowledge and modern tools and techniques in plant disease management in present days becomes easier to control this detrimental fungal pathogen. Due to this there is a wide level of variability in the pathogen and it exhibits low sensitivity against fungicide(s) or shows resistance/ tolerance to fungicides. A broad host range further helps it to acquire greater survival ability. These abilities not only pose serious threat in controlling the disease by chemical means but also cause great concern for its management by development of resistant varieties. Further, as a part of integrated disease management approach investigation by induction of SAR through the application of abiotic elicitors becomes a necessity. The crop protection and pest management through the use of biotic and abiotic elicitors is still in the very initial stages of use as a new plant disease control method and thus it is not yet in use at large scale in agricultural field. Keeping ecosystem and health hazards in mind, eco-friendly and alternate plant disease control method of crop plants using elicitor treatments with some advantages are like (i) reduced detrimental effects from fungi, herbivores, pests and insects, (ii) decreased level of environmental hazards and human health hazards as elicitors acts directly on the crop and vegetable plant and their acute toxicity is lower than chemical fungicides and pesticides to other living organisms, (iii) elicitors may be used along with the modern spraying technology as protective agrochemicals, (iv) elicitors treatments on plants could be an alternative to genetically modified (GM) crop plants to attract better natural enemies of pest on cultivated plants (Kappers *et al*.,2005) and (v) crop plants which were treated with biotic and abiotic elicitor have low level of ecological risks than genetically modified GM crops (Poppy and Wilkinson, 2005).

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