Plant Archives
Journal home page: www.plantarchives.org
DOI Url: https://doi.org/10.51470/PLANTARCHIVES.2021.v21.no1.129

IN- VITRO ASSAY OF ANTIFUNGAL ACTIVITY OF VARIOUS ELICITORS AND BINDERS AGAINST *ALTERNARIA ALTERNATA*

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(Date of Receiving-14-12-2020; Date of Acceptance-26-03-2021)

ABSTRACT

In the present study, *in-vitro* antifungal activity of various elicitors and binders were evaluated against *Alternaria alternata*, causative agent of early blight disease in Tomato. Antifungal activities were assayed by poison food technique.

Among all elicitors and binders best optimum activity was observed for castor oil cake and cow dung 63.06% and 60 .95% respectively against *Alternaria alternata*. On the basis of results obtained, best active elicitor i.e., castor oil cake and binder cow dung can be used to develop the plant extract-based bio-formulation for effective control of leaf blight disease of Tomato in an eco-friendly manner.

Keywords: Elicitors; Binders; Plant Extract; Bio-Formulation

INTRODUCTION

World widely, emphasis is increasingly being put on the healthy relationship between food, nutrition and health (WHO, 2004; WCRF, 2007). Therefore, the organic development of vegetables in sustainable manner not only increases the productivity and nutritional quality but also accomplish the challenges of population explosion and healthy and safe food. In global paradigm, India is the second largest producer of vegetables, producing 162.2 million tons supplying 14% of total world vegetable production (FAO, 2014 and Indian Horticulture Database, 2013). In 1991-92 the area occupied by vegetables in India was 5593 hectares that has attained and registered a quantum jump of 9396 hectares in 2013-2014 with the total production of 162897metric tons.

In India the most commonly cultivated vegetables are potato, cabbage, cucurbits, eggplant, okra, tomato, onion, carrot, chili and some other legumes (FAO, 2014). Vegetables are highly prone to the pathogens and continue assault make them susceptible to the disease and finally reduce the productivity of the crop. This all may be due the favourable climate that favours the reproduction. Among the major obstacles, pest and parasites occupy predominant position for restricting the productivity of the vegetables.

Genus *Alternaria* belongs to fungal class *deuteromycetes* having number of species and destructive plant pathogen to the plant families such as *Solanaceae*, *Cucurbitaceae*, and *Brassicaceae*. Tomato belongs to family *Solanaceae*. The tomato crop grown in rabbi and kharif season having nutritional and economical value. *Alternaria* species cause early blight disease in tomato and degrade the quality and quantity of crop. The disease is control by spraying synthetic chemicals but they are toxic to the environment, and create ecological problems. The application of biological materials to control the diseases, is eco-friendly alternative of chemical agents. Biological methods are safer, biodegradable and eco-friendly and cheaper for farmers rather than chemical control agents. Biological agents contain fungi, herbal extract and natural products are used to control diseases (Sachin Jadhav 2020).

The regulation of plant growth, development and alleviation of the negative effects of environmental stresses during ontogenesis, are important factors determining the productivity of cultivated plants. It is well recognized that biotic and abiotic stresses prevent them to achieve optimal growth and reduce their production (Yakhin *et al.,*2017). Today, in order to meet the growing demands of vigorous cultivation, application of chemicals has enormously been increased, which has resulted not only in the contamination of ground and surface water but also has interfered with soil biota. There are different options available for the farmers to prevent their crop from disease including use of resistant cultivars, crop rotation, tillage, biological control, and chemical pesticides. Effective chemical fungicides or pesticides worked on antibiotic principle, but their use at commercial level is too expensive and cumbersome in use. It has also been proved that excess use of chemical pesticides may be carcinogenic. Therefore, significant efforts have been made to develop environmental-friendly strategies for control of plant diseases (Hakeem *et al.,* 2015).

Up to now, extensive utilization of chemical pesticides, urbanization and industrialization have disrupted the ecosystem by contamination of the food chain. The species disturbance in the ecological equilibrium of the soil and water also unbalanced by persistent pesticide residues. It leads to reduced nutrient and flavour contents through low-cost intensive food production. Organic farming is an approach that has created attention and interest among the growers for raising healthy and safe food in sustainable

manner. The main aim of the organic farming is to provide nutritionally safe, potentially viable and high-quality food, and restore and maintain the long-term fertility.

In order to develop a new active bio-formulation as biological agent for control of plant disease screening is done to find out an active elicitor and binder which enhance the efficacy of plant extract. Initially the term elicitor was used for molecules that are capable of inducing the production of phytoalexins, but it is now normally used for any compounds that stimulate any kind of plant defence (Ebel J, Cosio EG 1994; Hahn MG1996; Nurnberger T1999; Kumari P *et al.,* 2018a) (Fig1). Elicitors include both substances originating from pathogen i.e., exogenous elicitors and compounds generated from plants, i.e., endogenous elicitors (Boller T.1995). Elicitors are also classified as biotic or abiotic, physical or chemical, and complex or depending on their origin and molecular structure. Several agro-based ravage and by products are known which play an important role in the management of crop plant diseases. They act directly or indirectly on plant pathogens and inhibit the growth and multiplication of the pathogen or induce resistance in crop. Some reports are available on use of agro based ravage and by product for control plant diseases like oil cakes (Kumari *et al.,* 2013; Sharma and Hada 2016; Meena M *et al.,* 2019b). Similarly, the larval hatching of *M. incognita* was suppressed significantly by boiled extracts of mustard and cotton oil-cake up to 99.92 and 99.38% in water. Eggs of M. *incognita* were found to be more vulnerable to oil-cakes (neem, karanj, mahua, groundnut, cotton, linseed, sesamum and kokam) and fungicide (Ceresan wet and Aureofungin-sol) treatment than larvae (Lanjeswar and Shukla, 1986). Neem cake extract was accomplished to be most potent in killing *M. incognita* larvae (Gowda and Setty, 1978; Gowda and Gowda, 1999) whereas, mustard cake extract proved to be most potent in manageing *Hoplolaimus indicus* (Deshmukh and Prasad, 1969). However, greater concentrations of oil-cake extract shown best results due to the presence of higher nematicide compound.

Binder is a material which holds other materials. Most of the natural polymers (gum and mucilage) are formed by high molecular weight carbohydrates. They are biodegradable, biocompatible and non-hazardous in nature and showed irregular physical-chemical properties and environmentally sustainable features (Goswami and Naik., 2014). Carbohydrates represent the most abundant biological molecules, covering a large array of fundamental roles in living things: from the reserve and transport of energy, (starch and glycogen), to the development of structural components (cellulose in plants, chitin in animals), and to the linking between intercellular walls (hemicellulose) (Kiranmoy Karmakar., 2016). Several materials such as guar gum, gum acacia etc. have been usedas binders. Guar gum, also known as guaran, is the ground endosperm of guar beans. In recent years, researches have used guar gum as a rate controlling mediator for controlled delivery of bioactive molecules (Sen *et al.,* 2010; Sarmah *et al.,* 2011; Angadi S.C. *et al.,* 2013; Yang H *et al.,* 2013). Similarly, Gum acacia has also been usedin traditional medicine and pharmaceutical industry but noone has reported till now for their application as binder in controlling plant disease (Dobelis1986; Meena M *et al.,* 2016 a, b).

Guar gum has a very strong hydrogen bond forming good tendency in water which makes it a novel thickener and stabilizer. Aqueous solutions of guar gum are very viscous in nature. Because of these properties it has wide applications in the industries like food, pharmaceutical, textile, oil, paint, paper, explosive and cosmetics (Morris, 1981; Mudgil *et al.,* 2014). Gum Acacia has astringent, emollient, liver tonic, antipyretic and antihistaminic property. It can be used as a binder up to 20% in concentration. Jwaraghanigutika were prepared with gum Acacia as it can add on the efficacy of it due to its antipyretic activity (Ali *et al.,* 2012; Kamini and Shuchi, 2016).

In India, cows are very Similarly, cow excreta (dung and urine) have shown antimicrobial activity from ancient times and have a holistic view in Hindu mythology. Rajeswari, *et al*. investigated the significant antifungal activity of cow dung extract against *Escherichia coli* and *Klebsiella pneumonia*. Krunal, *et al.,* also illustrated the significant activity of cow urine and cow dung against phytopathogenic fungus *Colletrotrichum falcatum*. In the present study, we tried to find out suitable binder and elicitor to get optimum antimicrobial efficiency from *Moringa oleifera* leaf extract.

MATERIALS AND METHODS

In the present study, antifungal activity of various elicitors and binders against *Alternaria alternata* was studied. For *in-vitro* antifungal activity there are seven type of elicitors i.e., ground nut oil cake, castor oil cake, mustard oil cake, cotton oilcake, sesame oil cake, coconut oil cake, and neem oil cake and four type of binders i.e., guar gum, gum acacia, coconut coir and cow dung were used.

In-Vitro Assay of Antifungal Activity of Various Elicitors and Binders Against *Alternaria alternata*

Seven elicitors and four binders were screened for antifungal activity against *Alternaria alternata*. 20 gm of each elicitor was dissolved in 100 ml of autoclaved water for 24 h. The mixture was then filtered and the filtrate was further used for antifungal activity. The antifungal activity of each elicitor was tested using poison food technique (Ref). 1 ml of each elicitor with 9 ml molten sterile PDA culture medium was poured into pre-sterilized Petri plates (9 cm diameters) and allowed to solidify at room temperature. Thus, prepared petri-plates were inoculated aseptically with 6mm disc of test pathogen *Alternaria alternate* cultures which was placed at the centre of the plate. The Petri-plates were then incubated at 28 ± 2ºC for seven days, only PDA culture media are used as control series. Antifungal activity of each elicitor and binder was measured as a function of increase in growth diameter of 6 mm disc of inoculum. The experiment was conducted

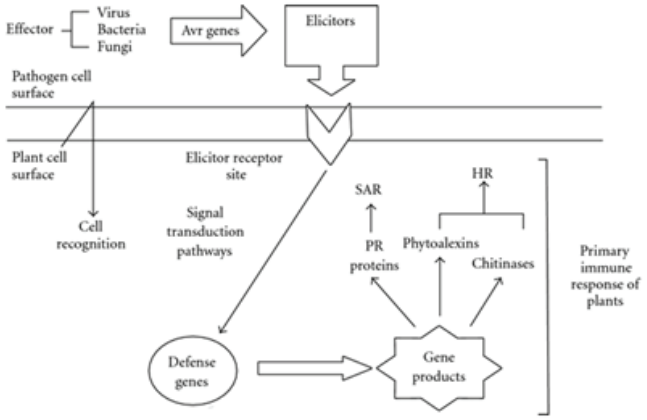


Figure-1: Mechanism of Elicitors Action

in replicates. The growth inhibition was measured as per formula given below

Mycelial (%) growth inhibition= gc-gt ×100

gc = growth of fungal colony after 7 days incubation period in control set subtracting the diameter of inoculums disc.

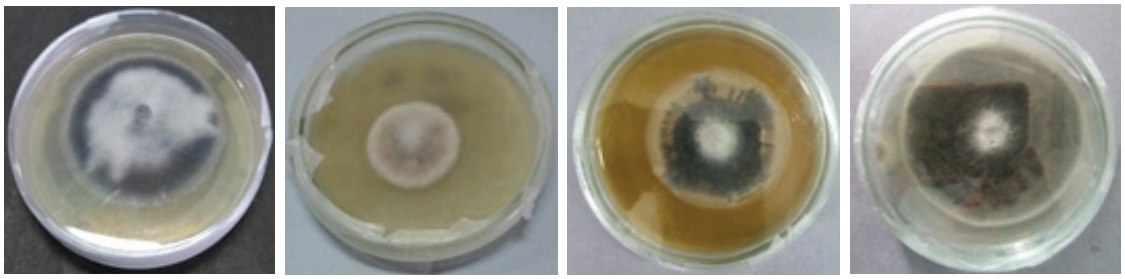
gt = growth of fungal colony after 7 days incubation period in treatment set subtracting the diameter of inoculums disc.

RESULTS AND DISCUSSION

Seven types of elicitor and four types of binder were screened for their antifungal activity against *Alterneria alternata* by food poisoning assay. After seven days of incubation the antifungal activity was measured. The results of antifungal activity of various elicitors against *Alternaria alternata* are given in Table 1 and Fig1A. Among the all-elicitors castor oil cake showed best activity with. 63.06% inhibition followed by mustard-oil cake with58.93% inhibition (Table1) The results of antifungal activity of various binders against *Alrenaria alternate* shown in fig*2* Among the all binders the best activity was observed in cow dung extract with60.95% inhibition followed by coconut coir and guar gum54.86% and 36.17%, respectively (Table2).

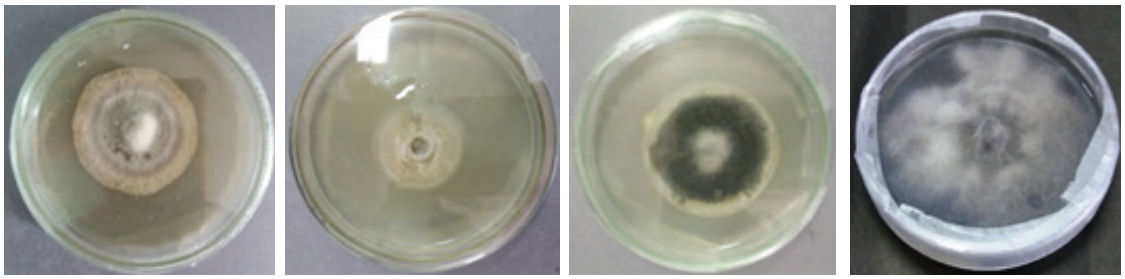
Plant extracts have been used for centuries in medicine and pest control (Islam *et al.,* 2004; Ashraf *et al.,* 2011; Adhikari *et al.,* 2017). Use and exploitation of preparations based on natural products, have pesticidal activity comes into higher and higher prominence, especially limiting synthetic fungicide/ traditional chemical application. It results from equivalent efficiency of bio-preparations to pesticides.

It was observed that oil cake showed concentration dependent antimicrobial activity. The same pattern was also reported by Kuo, 1967; Gohl,1970; Ahamed and Mollah, 1992; Tiyagi and Alam, 1995; Shukla and Haseeb,1996; Khan and Saxena, 1997; Omar, 2002; Rao *et al.,* 2003; Jothi *et al.,* 2004.Oil cakes are by products obtained after oil extraction from the seeds. The efficacy of oil seed cakes of neem, castor and mustard against plant parasitic nematodes and soil inhabiting fungi infesting mung bean and the subsequent crop, chickpea was investigated. The population of plant-parasitic nematodes, *Meloidogyne incognita*, *Rotylenchulus reniformis*, *Tylenchorhynchus brassicae,* and *Helicotylenchus indicus*, and the frequency of the pathogenic fungi *Macrophomina phaseolina*, *Rhizoctonia solani, Phyllosticta phaseolina*, *Fusarium oxysporum*, etc., were significantly reduced by these treatments



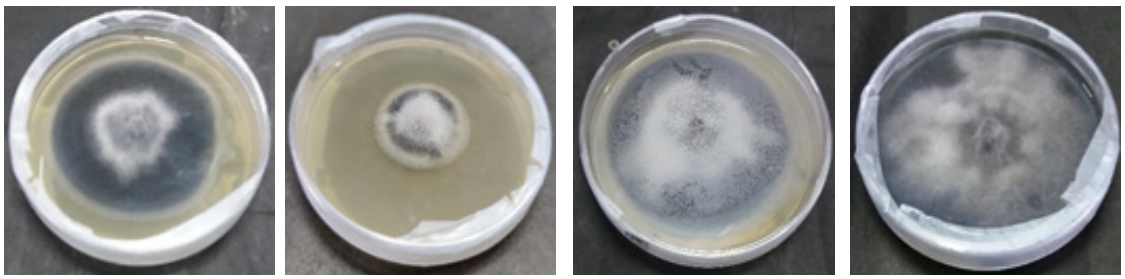
Cotton Oil Cake | Mustard Oil Cake | Neem Oil Cake | Sesame Oil Cake

Figure-2: Mycelial growth in different Elicitors



Groundnut Oil | Castor Oil Cake | Coconut Oil Cake | Control

Figure-3: Mycelial growth in different Elicitors



Guar gum | Cow dunk | Acacia gum | Control

Figure-4: Mycelial growth in different Elicitors

Table1: Antifungal Activity of Oil Cakes against *Alrenaria alternate*

S. No.	Elicitors	Growth diameter after 7 days (mm) ± SD	Percentage (%) mycelial growth inhibition
1	Neem oil cake	39.42	50.03
2	Mustard oil cake	32.4	58.93
3	Cotton oil cake	40.66	48.46
4	Castor oil cake	29.14	63.06
5	Coconut oil cake	38.56	51.12
6	Ground nut oil cake	36.33	53.95
7	Control	78.90	-

Table2: Antifungal Activity of Binders Against *Alrenaria alternate*

S. No.	Elicitors	Growth diameter after 7 days (mm) ± SD	Percentage (%) mycelial growth inhibition
1	Cow dung	30.81	60.95
2	Guar gum	50.36	36.17
3	Acacia gum	60.53	23.28
4	Coconut coir	35.61	54.86
5	Control	78.90	-

(Tiyagiand Alam, 1995).

Oil cakes have also been reported for use in production of antibiotics and antimicrobials. Oil cakes in mixture of 35% wheat bran, 20% mustard oil cake (MOC), 25% cow dung and 20% fine sand were described for tubificid worm's production in a culvert system under running water. New offspring appeared after20 days from the start of the experiment, and 2.85 g raw materials produced 1.0 g of worms (Ahamed and Mollah, 1992). Oil cakes in combination with Bradyrhizobium sp. and Paecilomyceslilacinus have been studied for control of root rot of mungbean (Ehteshamul-Haque *et al.,* 1995).

Khan and Saxena (1997) reported that improvement in tomato plant growth with reduced nematode growth in neem cake amended soil. Similar study using some nematicides (aldicarb, carbofuran, ethoprop) along with oil cakes (linseed, mustard, neem) against *Pratylenchus thornei* infesting *Mentha citrata, M. piperita* and *M. spicata* in glasshouse experiments has been reported and find out Neem cake was most effective in reducing the reproduction rate of *P. thornei* and improved the herb weight and oil yield, followed by mustard cake, aldicarb, ethoprop, carbofuran and linseed cake, correspondingly. (Shuklaand Haseeb, 1996). Also, use of oil cakes of neem, castor and mahua independently and in combination with a chemical nematicide (carbofuran 3G) for the management of *Pratylenchus delattrei* in crossandra under glass house conditions has been reported. Neem oil cake was effective compared to other oilcakes used and there was a synergistic effect when the neem cake was coupled with carbofuran 3G in the management of *P. delattrei* (Jothi *et al.,* 2004).

Our results indicate that binders also have antimicrobial activity but lower than oil cakes. Among the all binders like cow dung, guar gum and gum Acacia,cow dung shows best inhibition against *Alternaria alternata.* Farmers in India use neem leaves to protect their stored grain from insects. Herbs and spices, such asbasil and clove, have also been used by many workers to protect food from spoilage, as both have antimicrobial properties (Manohar *et al.,* 2001). Matsuzaki *et al.,* (1998) investigated that soil amended with cow manure is ideal treatment for decreasing the severity of the disease and improving the final tubers yield of potato.

Combination of cow dung and urine with *Vinca rosea*, *Piper betle* and *Azadiracta indica* extracts leads to 100% inhibition of conidial germination of leaf blight causing fungi in wheat plant (Nargis *et al.,* 2006). Cow dung also contains an antifungal substance which inhibits the growth of corprophilous fungi (Nina *et al.,* 2006). *Allium sativum* raised on cow dung slurry exhibited the highest zone of inhibition with spore germination of the fungi (Yongabi *et al.,* 2009; Joseph and Sankarganesh, 2011). Basak and Lee (2002) reported that fresh cow urine and cow dung has positive comeback in suppression of mycelial growth of *F. solani, F. oxysporum* and *S. sclerotiorum*. coconut coir is used in the production of ropes, mattress padding and brushes, and constitute 30% of the coir, while the remaining 70% consists of coir dust (van Dam, van den Oever, Teunissen, Keijsers, & Peralta, 2004). These particulate materials are characteristically used for agricultural proposes, such as a material to fertilize the soil, increase the porosity of the soil and retain water. However, a large amount of this waste is incinerated without any control of gas emissions, and it therefore constitutes a source of contamination (Abad, Noguera, Puchades, Maquieira, & Noguera, 2002). Coir dust mainly consists of hemicellulose (29.5wt%), lignin (24.1wt%) and α-cellulose (21.0%) (van Dam, *et al.,* 2004). It has a highly porous structure and has a low susceptibility to biodegradation, which makes it suitable for holding huge quantities of water (more than 50% by weight) (Ohler, 1984).Harish, *et al.,* screened four oil cakes i.e. mahua cake extract, neem cake extract, castor and gingelly cake extracts contrary to Bipolarisoryzae, the fundamental agent of brown spot disease in rice and amongst the all cake extracts, neem cake extract showed the maximum inhibition of mycelial growth and spore germination followed by mahua cake extract, castor and gingelly cake extract. Coventry &Allan illustrated the antimicrobial effect of neem seed extract against different plant pathogens. Singh, *et al.,* reported that foliar spray of aqueous extract of neem cake showed significant antifungal potential against powdery mildew of balsam. The antimicrobial properties of gossypol from cotton plant have been reported by several researchers. Barupal *et al.,* (2020) also evaluated the antifungal activity of

guar gum, gum acacia and cow dung against *Curvularia lunata* and best antifungal activity was observed with cow dung followed by gum acacia, guar gum. Gum acacia (galactoaraban) is obtained from tree plant *Acacia nil.* In this experiment we find out best antifungal activity shown by castor oil cake followed by mustard oil cake, ground nut oil cake, coconut oil cake, neem oil cake and among the all binders the best activity was observed in cow dung followed by coco coir, guar gum and acacia gum.

CONCLUSION

In plant biology elicitors are extrinsic, or foreign, molecules often associated with diseases or synergistic organisms and plant pests. Molecules of elicitor can attribute to precise receptor proteins positioned on plant cell membranes. The molecular pattern of elicitors is recognized by receptors and trigger intracellular defence signalling via the Octadecanoid pathway. The response results in the enhanced synthesis of metabolites which decrease damage and increase resistance to pest, disease or environmental stress (Bektas & Eulgem, 2015). Binder is a material which holds or draws other constituents together to form a cohesive whole mechanically, chemically, or as an adhesive. Generally, materials labeled as binders in various proportions or uses can have their roles reversed with what they are binding. The material is used by Egyptians as glue and as a pain-reliever base. Arabic physicians with the gum treated a wide variety of ailments, resulting in its current name (Dobelis, 1986). Presently, it issued widely in the cooking industry to give body and texture to processed food products and in the pharmaceutical industry as a demulcent. It is also used to stabilize emulsions. The bark fibers are used to make cordage (Duke, 1985).

Current study indicated that castor oil cake as elicitor and cow dung as binder shown maximum antimicrobial activity against *Alternaraia alternate*, an early blight pathogen of tomato. Cow dung and castor oil can be used to develop the plant extract-based bio-formulation for effective control of early blight disease of tomato in an eco-friendly manner. These bio-formulations could be considerable in the direction of sustainable agriculture without destroying biological system. Such manufactured bio-formulations would have vast potential to be economically explored for agriculture use.

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