



# HERBAL BIO-FORMULATION : AN EFFECTIVE MEAN TO CONTROL *ALTERNERIA SOLANI*

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## Abstract

Herbal bio-formulations which are based on natural products are best alternative of existing chemical fungicides. These plant based formulation do not have any residual effect. In the present study attempt has been made to develop formulation containing combination of elicitor, binder and plant extract. Presence of elicitors and binders not only enhance antifungal activity but also field stability of formulation. Total 30 combinations of formulations were prepared, among these combinations, no. 7 and 13 prepared with crude showed best antifungal activity *i.e.* 79.34% and 78.10% mycelial growth inhibition respectively. In rest of preparations which involves partially purified plant extract, maximum mycelia growth inhibition was observed with preparation no 18 and 23 *i.e.* 85.54% and 83.06% respectively. Thus prepared formulation effectively inhibits the growth of *Alternaria solani*. Hence, this formulation can be used to manage crop and plant losses caused by fungal pathogens.

**Key words:** Herbal bio-formulations, natural products, fungal pathogens, growth inhibition, elicitors, binders, antifungal.

## Introduction

Enormous array of secondary metabolites are produced by plants and this chemical diversity protect the plant from several plant pathogenic fungus causes severe economic losses (Ghosh *et al.*, 2013). An investigation of alternative strategies for the control of pests and pathogens is very important to control environmental pollution (Lyon *et al.*, 1995). Plant products/extracts have found to be effective over conventional fungicides and microbial bio-control agents, to control wide range of pathogens (Bowers and Locke, 2004). Furthermore, Herbal bio-formulations based on plant extract, are systemic, specific in action, nonphytotoxic, cost effective and have poor environmental retention (Singh, 1994). Herbal formulation is a balanced composition involves suitable elicitors and effective binders with plant extract. Addition of elicitors allows getting maximum benefits of naturally present phytochemicals in selected plant extract. In order to reduce the cost of developed formulation use of appropriate and cheap elicitors and binders is prime requirement. Oil cakes like neem oil cake are some easily available elicitors usually individually incorporated in any formulation. Elicitors have property to elicit the plant growth by providing some essential nutrients and in combination with plant extract,

elicitors strengthen the plant or crop to fight with fungal pathogens (Juveriya and Ahmmed, 2006). One of the important content needs to be added in herbal formulations is binders. Some naturally available binders usually incorporated in herbal formulation are Guar gum, gum acacia and cow dung. These are the natural organic fertilizers with high nitrogen content and left over part after oil extraction process. Recent trends towards the use of plant based and natural products demand the replacement of synthetic additives with natural ones. These natural materials have many advantages over synthetic ones as they are chemically inert, nontoxic, less expensive, biodegradable and widely available. In order to develop formulations it is very important to add elicitors and binders to plant extract which not only add to antifungal property of used extract but also improve its field stability. Antimicrobial traits of plant extract are due to the antimicrobial compounds synthesized in the secondary metabolism of the plant. These products are known by their active substances, for example, saponin, alkaloid, steroid, flavonoid, tannin, phenol and carbohydrate compounds which are the major part of various plant extracts. Organic amendments are generally used for improving crops, increasing agricultural productivity and suppressing soil borne diseases (Stone *et al.*, 2003). Beside a wide variety of organic matters that have been

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**Table 1:** Antifungal activity of various elicitors against *A. solani*.

S. No.	Elicitors	Growth Diameter after 7 days (mm) ± SD	% Mycelial growth inhibition
1.	Neem oil cake	32±1.00	60.32
2.	Mustard oil cake	41±1.00	49.16
3.	Cotton oil cake	43±1.00	46.68
4.	Sessame oil cake	47±1.00	41.73
5.	Ground nut oil cake	41±1.00	49.16
6.	Coconut oil cake	45±1.00	44.21

tested as organic amendments for managing plant pathogens are oil seed cakes which decreased the population of soilborne pathogens (Sharma *et al.*, 1995). Oil seed cakes are by-products obtained after oil extraction from the seeds. Oil cakes are of two types, edible and non-edible. Non-edible oil cakes such as castor cake, neem cake are used as organic nitrogenous fertilizers, due to their NPK content. Some of these oil cakes are found to increase the nitrogen uptake of the plant and protect the plants from soil nematodes, insects and parasites (Ramachandran *et al.*, 2007). It is observed that several antimicrobial by-products (*e.g.* organic acids, hydrogen sulfide, phenols, tannins and nitrogenous compounds) are released during the decomposition of organic amendments, or synthesized by microorganisms involved in such degradation (Rodriguez-Kabana *et al.*, 1995). Cow dung is the undigested residue of plant matter which has passed through the animal's gut. The resultant faecal matter is rich in minerals. Cow dung is comprised of organic matter including fibrous material that passed through the cow's digestive system, among other liquid digesta that has been left after the fermentation, absorption and filtration, then acidified, then absorbed again (Rajeswari *et al.*, 2016). Cow dung also known for its antifungal activity (Waziri *et al.*, 2013). Thus present study describes about the antifungal activity of some selected binders and elicitors so that effective herbal formulation can be prepared against test pathogen.

## Materials and Methods

### Preparation of Herbal Formulation

Herbal formulations were prepared by using plant extracts (100% alcohol extract and partially purified fraction of alcohol, Elicitor (neem oil cake) and Binder (gum acacia) in different ratio (Table 1). Total number of 30 bioformulation were prepared in which formulation no. 1 to 15 were made by using 100% alcohol crude extract and formulation ration number 16 to 30 were made by using partially purified alcohol extract its combination with elicitor and binder.

**Table 2:** Antifungal activity of various Binders against *A. solani*.

S. No.	Binders	Growth Diameter after 7 days (mm) ± SD	% Mycelial growth inhibition
1.	Cow dung	51.33±0.577	36.36
2.	Guar gum	57.33±0.577	28.92
3.	Gum acacia	60.33±0.577	25.20
4.	Control	80.66±0.577	-

### *In vitro* Antifungal Activity of Herbal Formulations

*In vitro* antifungal activity of bioformulations against *Alternaria solani* was tested using poison food technique (Groover and Moore *et al.*, 1962). 1 ml of each formulation was mixed with the 9 ml of molten sterile potato dextrose agar culture medium and this mixture was poured into the pre-sterilized petri-plates (9 cm diameters) and allowed to the solidify at the room temperature. Only PDA culture media was used as control series along with the test samples. The prepared petri-plates were then inoculated aseptically with 6 mm disc of the test pathogens culture and incubated at 28±2°C for seven days. After seven day of the incubation, average diameter of fungal colonies was measured and percent mycelial growth inhibition was calculated by the following formula:

$$\text{Mycelial growth inhibition} = \frac{gc - gt}{gc} \times 100$$

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

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

## Results and Discussion

It was observed that among the elicitors included in these study maximum percent mycelial inhibition was observed with neem oil cake *i.e.* 60.32% followed by mustard oil cake and groundnut oil cake *i.e.* 49.16% and 49.16% respectively. In case of binders, maximum inhibition was observed with cow dung *i.e.* 36.36 % while least was observed for gum acacia *i.e.* 25.20% (Table 1 and 2).

Results of antifungal activity of herbal formulations consist of different combinations of elicitors, binders and plant extract against *Alternaria solani* given in table 3. By mixing varying quantity of elicitors, binders and plant extract *i.e.* neem oil cake, cow dung and crude as well as partially purified extract respectively, 30 different

**Table 3:** *In Vitro* Antifungal Activity of Herbal Formulation against *Alternaria solani*.

Formulation No.	Formulation type	Ratio	Growth Diameter after 7days (mm) $\pm$ SD	% Mycelial Growth Inhibition
1.	100% alcoholic crude: Neem oil cake: cow dung	1:1:8	33 $\pm$ 1	59.08%
2.	100% alcoholic crude: Neem oil cake: cow dung	2:2:6	21 $\pm$ 1	73.96%
3.	100% alcoholic crude: Neem oil cake: cow dung	3:3:4	27 $\pm$ 1	66.52%
4.	100% alcoholic crude: Neem oil cake: cow dung	4:4:2	31 $\pm$ 1	61.56%
5.	100% alcoholic crude: Neem oil cake: cow dung	5:3:2	29 $\pm$ 1	64.04%
6.	100% alcoholic crude: Neem oil cake: cow dung	8:1:1	22 $\pm$ 1	72.72%
7.	100% alcoholic crude: Neem oil cake: cow dung	6:2:2	16.66 $\pm$ 0.577	79.34%
8.	100% alcoholic crude: Neem oil cake: cow dung	4:3:3	29.66 $\pm$ 0.577	63.22%
9.	100% alcoholic crude: Neem oil cake: cow dung	2:4:4	29 $\pm$ 1	64.04%
10.	100% alcoholic crude: Neem oil cake: cow dung	2:5:3	28 $\pm$ 1	65.28%
11.	100% alcoholic crude: Neem oil cake: cow dung	1:8:1	29 $\pm$ 1	64.04%
12.	100% alcoholic crude: Neem oil cake: cow dung	2:6:2	30 $\pm$ 1	62.80%
13.	100% alcoholic crude: Neem oil cake: cow dung	3:4:3	17.66 $\pm$ 0.577	78.10%
14.	100% alcoholic crude: Neem oil cake: cow dung	4:2:4	30 $\pm$ 1	62.80%
15.	100% alcoholic crude: Neem oil cake: cow dung	3:2:5	30 $\pm$ 1	62.80%
16.	Partially purified Alcohol extract:Neem oil cake:cow dung	1:1:8	36 $\pm$ 1	55.36%
17.	Partially purified Alcohol extract:Neem oil cake:cow dung	2:2:6	33.66 $\pm$ 0.577	58.26%
18.	Partially purified Alcohol extract:Neem oil cake:cow dung	3:3:4	11.66 $\pm$ 0.577	85.54%
19.	Partially purified Alcohol extract:Neem oil cake:cow dung	4:4:2	33.66 $\pm$ 0.577	58.26%
20.	Partially purified Alcohol extract:N.eem oil cake:cow dung	5:3:2	33 $\pm$ 1	59.08%
21.	Partially purified Alcohol extract:Neem oil cake:cow dung	8:1:1	32 $\pm$ 1	60.32%
22.	Partially purified Alcohol extract:Neem oil cake:cow dung	6:2:2	33 $\pm$ 1	59.08%
23.	Partially purified Alcohol extract:Neem oil cake:cow dung	4:3:3	13.66 $\pm$ 0.577	83.06%
24.	Partially purified Alcohol extract:Neem oil cake:cow dung	2:4:4	34.66 $\pm$ 0.577	57.02%
25.	Partially purified Alcohol extract:Neem oil cake:cow dung	2:5:3	36.66 $\pm$ 0.577	54.54%
26.	Partially purified Alcohol extract:Neem oil cake:cow dung	1:8:1	36 $\pm$ 1	55.36%
27.	Partially purified Alcohol extract:Neem oil cake:cow dung	2:6:2	35.66 $\pm$ 0.577	55.78%
28.	Partially purified Alcohol extract:Neem oil cake:cow dung	3:4:3	34.66 $\pm$ 0.577	57.02%
29.	Partially purified Alcohol extract:Neem oil cake:cow dung	4:2:4	35 $\pm$ 1	56.60%
30.	Partially purified Alcohol extract:Neem oil cake:cow dung	3:2:5	36 $\pm$ 1	55.36%
–	Control		80.66 $\pm$ 0.577	NI

herbal formulations differ in composition was obtained. Among the formulations prepared, herbal formulation no. 1-15 contained 100% alcoholic crude extract whereas formulation no. 16-30 contained alcohol fraction of plant extract. Among the 1-15 combinations prepared using crude extract, best antifungal activity was observed for no. 7 and 13 *i.e.* 79.34% and 78.10% mycelial growth inhibition respectively. In rest of preparations which

involves partially purified plant extract, maximum mycelia growth inhibition was observed with preparation no 18 and 23 *i.e.* 85.54% and 83.06% respectively.

### Discussion

In order to conserve and establish an agricultural system with ecological sustainability, it is necessary to develop biological methods to not only control the fungal

infections in crop and plants, but also to ensure productivity, quality of plants and to reduce environmental pollution. Herbal formulations formulated by combination of elicitors, binders and plant extract are one of the best ecofriendly approach to control fungal disease. Elicitors and binders complement the efficacy of plant extract which alone can be very expensive. Currently, binders are included in novel dosage forms to fulfil specific functions and in some cases they directly or indirectly influence the extent and/or rate of release and action of plant extract. One of the possibilities involves, use of herbal formulations, usually prepared by using plant extracts are best alternative of existing chemical control methods. These natural formulations not only effective in control diseases losses of crop but also provide nutrients to plant. Excessive use of fungicides have decrease the soil fertility hence need of hours is to develop natural remedies which not only protect the plant from microbial diseases but also maintain the fertility of soil. Thus, there is a growing interest on the research on the possible use of natural products such as plant based essential oils and extracts, which may be less damaging for fungal disease control. Plants have long been recognized to provide a potential source of chemical compounds or more commonly products, known as phytochemicals, which include essential oil and plant extracts. Research into plant derived fungicides for their possible applications to control plant pathogenic fungi is being intensified as these have enormous potential to inspire and influence modern agrochemical research (Bajpai and Kang, 2012). Natural elicitors like ground nut oil cake, mustard oil cake, cotton oil cake, sesame oil cake, coconut oil cake and neem oil cake and binders like cow dung, guar gum and gum acacia have been observed for antifungal activity. The commercial value of oil cakes depends on water and fat content present. Moisture usually accounts for between 20 and 30 percent of the weight, while the fat content varies from a minimum of about 2.5 percent of the weight to 10-12 percent and in some cases even more, depending on the extraction process used. In addition to its use as stock feed for animals, the residue can also be used as a fertilizer, after it has been properly steeped to avoid fermentation, which would damage the plants (Al-Momany and Al-Saket, 1989). Among the oil cakes under study, neem oil cake observed to be efficiently inhibiting the mycelial growth of *A. solani*. Binders like guar gum and gum acacia are natural plant products complement the antifungal efficacy of plant extract. Mostly nontoxic for human system, show limited field persistence and have no residual threats. Cow dung is comprised of organic matter including fibrous material that passed through the

cow's digestive system, among other liquid digesta that has been left after the fermentation, absorption and filtration, then acidified, then absorbed again. The chemical composition mostly carbon, nitrogen, hydrogen, oxygen, phosphorus, etc. with salts, cells sloughed off as the digest went through the digestive tract, some urea, mucus, as well as cellulose, lignin and hemicelluloses (Rajeswari *et al.*, 2016). Results of antifungal activity of herbal formulations demonstrated that among the formulations prepared from 100% alcoholic crude extract and partially purified alcoholic fraction of *Thevetia* leaves, 6:2:2 proportion of 100% alcoholic crude:Neem oil cake:cow dung and 3:3:4 proportion of Alcohol extract:Neem oil cake:cow dung observed to be best against *Alternaria solani* causing early blight of potato disease. These results confirmed that plant extracts originated from invasive plants can be used with elicitors like neem oil and binders like gum acacia to develop new and effective classes of natural fungicides to control severe fungal diseases. Several researchers studied that preparation of formulations from the botanicals showing food antimicrobial activity could progress the adoption of ecofriendly disease management methods by the farmers (Kumbhar *et al.*, 1999; Sharma *et al.*, 1999; Stompor - Chrzan, 2003). Preliminary work on development of formulations in the form of emulsifiable concentrates (EC), dusts (D) and wettable powder (WP) from plant extracts showed promise for disease management (Veerasingam, 1997; Chandrasekaran, 1996; Rajappan *et al.*, 1999). Commercial botanical fungicides (Wanis) developed from plant based monoterpenes were also used for management of *Fusarium sp.* (Narashimhan *et al.*, 1999). But these botanical fungicides have not been widely tested for their efficacy in vegetables. Hence effective proportion of formulation prepared using crude and partially purified preparations of leaf extract of *Thevetia peruviana*, studied in present work could be very useful to control economical loss causes by fungal pathogen *Alternaria solani*.

## Conclusion

Thus formulation based on natural elicitor, binders and plant extract leads good inhibition of fungal pathogen causes significant losses in economically important crops like potato. After effective field trials these formulations can be prove better alternative of available chemical control methods causing negative effect on environment as well as human health.

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