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# *IN VIVO* MANAGEMENT OF EARLY BLIGHT DISEASE OF POTATO WITH HERBAL FORMULATION

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## ABSTRACT

In the present study investigates the *in vivo* management of early blight disease of potato by seed dipping and foliar spray with herbal formulation from *Cassia fistula* L. fruit pulp extract in combination with neem oil cake and cow dung. The preventive action was studied as a function of decrease in disease severity, change in growth characteristics of host plant such as number of leaves/plant, plant height, number of tubers, tuber weight, and tuber size of healthy, infected and treated plants. Six treatments i.e. T1, T2, T3, T4, T5 and T6 were applied in different combinations. Four different controls i.e. C1, C2, C3 and C4 were also maintained. Results suggested that T4 treatment not only reduces the infection but also leads to increased growth, health and vigour of the host plant as compare to other treatments. This combination observed to show significant activity against *Alternaria solani* and can help to minimize the economical loss of potato crop. Data were subjected to analysis of CD and CV value and statistical analysis at 5% and 1% CD revealed that all the treatments are significant.

**KEYWORDS:** Herbal formulation, neem oil cake, cow dung, potato, *Alternaria solani.*

## INTRODUCTION

Potato (*Solanum tuberosum* L.) is an annual, herbaceous plant of family Solanaceae. Although, it originated in South America in the region between Peru and Bolivia in the 16th century, it is now a globally important crop, exceeded only by maize, rice and wheat and is consumed by billions of people across the globe, half of which are in the developing countries. It is the fourth important crop worldwide by volume of production. It is high yielding, has a high nutritive value and is grown in about 140 countries.[1] The present area under potato cultivation in India is about 1.4 million hectares. India produces a total of about

25-28 million tones of potatoes every year.[2] Potato is an economical food and a source of low cost energy to the human diet. It is also used for production of high quality starch, alcohol, etc. and its starch (farina) is used in laundries for sizing yarn in textile mills. It is also used for the production of dextrin and glucose. Potato tubers contain about 77.8% water, 22.6% carbohydrates, 2.1 % protein, 0.3% fat, 1.1% crude fibre, 0.9% ash, 40 IU vitamin A, 12 mg ascorbic acid per 100 g of edible portion etc. This crop is highly susceptible to early blight caused by *Alternaria solani*.[2]

Early blight is a very common disease of both potato and tomato. It is caused by the fungus, *Alternaria solani* (Ellis & G. Martin) L.R. Jones & Grout, which survives in infected leaf or stem tissues on or in the soil. Spores form on infested plant debris at the soil surface or on active lesions over a fairly wide temperature range, especially under alternating wet and dry conditions. The pathogen can also attack potato tubers and symptoms are circular to irregular lesions that are slightly sunken and often surrounded by a raised purple to dark brown border and produce a shallow, dry, corky rot.[4,5]

Estimating total annual crop losses due to any particular disease is difficult to do accurately. Yield losses up to 79 per cent from early blight damage have been reported from India.[3,6] Apart from the use of crop rotation, certified disease free seeds and resistant varieties, and control measures are important to minimize infection. It is usually necessary to apply fungicide sprays to fully protect plants from early blight. Fungicide alternatives that have been fungicidal effect on disease incidence.

The overzealous and indiscriminate use of most of the synthetic fungicides has created different types of environmental and toxicological problems. Bavistin, mancozeb and thiram are the most commonly used plant fungicides. Such synthetic fungicides bring about the inhibition of pathogens by either destroying their cell membrane or its permeability or by inhibiting metabolic processes of the pathogens and hence are extremely effective.[7] The flip side of this is that synthetic chemicals are harmful for human as well as soil health. They decrease soil fertility, enter the food chain, pollute the environment and cause several deleterious effects on human health and biosphere, contributing to significant declines in populations of beneficial soil organisms, soil acidification and compaction, thatch accumulation, and diminished resistance to diseases.[8] Use of alternative methods which are sustainable and eco-friendly is therefore the answer to this problem.

Thus, current thinking about plant and environment protection suggests alternatives to pesticides and use of other strategies in addition to well known disease management methods such as crop rotation use of resistant cultivars, planting disease free seeds, biological control etc. for control of fungal diseases.[9,10] One of these alternative methods is use of natural formulations prepared from plants. Plants possess natural antimicrobial compounds (which can be used to develop eco-friendly and effective fungicidal formulations). Although several plants extracts have been screened for in vitro antifungal activity against plant pathogenic fungi.[11,12] But not much work has been done on *in vivo* study of preventive or therapeutic effect of plant extracts.

The present study investigates the *in vivo* management of early blight disease of potato by seed dipping and foliar spray with herbal formulation from *Cassia fistula* L. fruit pulp extract in combination with neem oil cake and cow dung. A comparison with synthetic fungicide has also been done. Innovative part of this study is use of combinations of plant extract with elicitors and binders to develop protective measure against this plant disease. The preventive action was studied as a function of decrease in disease severity, change in growth characteristics of host plant such as number of leaves/plant, plant height, number of tubers, tuber weight, and tuber size of healthy, infected and treated plants.

## MATERIALS AND METHODS

**Test crop and fungus:** *Alternaria solani* was isolated from infected leaves of potato collected from the nearby fields of Udaipur (Saveenakheda, Manwakheda etc). Identification of fungus was done by Dr. T. Prameela Devi (Head, Division of Plant Pathology, Indian Type Culture Collection, IARI, New Delhi with I.D. No. 9944.15). Potato tubers were procured from the local market of Udaipur. Healthy seed tubers of approximately same size and having 3-5 eyes were used for experiment.

**Inoculum development:** Potato dextrose agar (PDA) medium which was sterilized in an autoclave at 15 psi for 20 minutes was used for culture of *Alternaria solani*. The inoculated petriplates were incubated at $25^0$ C for seven days. Pure culture thus obtained was used for mass culture of *Alternaria solani*.

Mass culture of *Alternaria solani* was done in 250 ml flasks containing 25 ml of autoclaved potato dextrose broth. Each flask was inoculated with 6 mm diameter of the fungus taken from the margins of a week old culture of *Alternaria solani*, grown on PDA medium. Then

flasks were incubated at 25 ± 20c for 72h. 25 ml of *Alternaria solani* suspension per pot of soil was used as inoculum for experimentation.

**Preparation of pots and soil:** Pots (30 cm Diameter) were sterilized with 20% CuSO4 solution whereas soil was autoclaved and then cooled. Sterile soil was mixed with inoculum and filled in the pre-sterilized pots. Since autoclaving of soil makes it nutrient deficient hence organic manure was added to the soil of each pot before sowing.[13]

**Preparation of herbal formulations and standard solution**

*In vivo* study of preventive /protective action of herbal formulation based on results of *in vitro* studies. Herbal formulations were prepared by using *Cassia fistula* fruit pulp extracts, powder, elicitor and binder in different ratio. 100% of neem oil cake and 100% of cow dung were mix with 100% alcoholic crude, partially purified chloroform extract and fruit pulp powder. All ingredients of herbal formulation were used in following combinations:

- Crude extract + elicitor + binder
- Partially purified extract+ elicitor + binder
- Fruit pulp powder + elicitor + binder.

Mancozeb was used as standard antifungal and 10 mg/ml concentration was prepared in sterile water.

**Treatments and experimental design:** The study was conducted during the December, 2015 to March 2016 in the Botanical Garden, University College of Science, M.L.S. University, Udaipur, (Rajasthan). Seed dipping method and foliar spray were used for the study of preventive effect of herbal formulation.[14,15]

On the basis of results obtained from *in vitro* studies six treatments were applied in following combinations.

T1: Healthy tubers were treated with formulation no. 8 (100% alcoholic crude extract (4ml): 100% neem oil cake (3ml): 100% cow dung (3ml)).

T2: Healthy tubers were treated with formulation no. 12 (100% alcoholic crude extract (2ml): 100% neem oil cake (6ml): 100% cow dung (2ml)).

T3: Healthy tubers were treated with formulation no. 18 (Partially purified chloroform extract (3ml): 100% neem oil cake (3ml): 100% cow dung (4ml)).

T4: Healthy tubers were treated with formulation no. 22 (Partially purified chloroform extract (6ml): 100% neem oil cake (2ml): 100% cow dung (2ml).
T5: Healthy tubers were treated with fruit pulp powder (60gm): neem oil cake (20gm): cow dung (20gm).
T6: Healthy tubers were treated with fruit pulp powder (40gm): neem oil cake (30gm): cow dung (30gm).

Healthy seed tubers dipped in respective herbal formulations were planted in pre-sterilized soil pots containing 10 kg soil infested with *Alternaria solani* inoculum showed in Fig.7 & 8.

Four different controls were also maintained respectively showed in Fig. 7. These were as follows
C1: Healthy tubers were treated with 10mg/ml concentration of Mancozeb sown in soil inoculated with *Alternaria solani*.
C2: Untreated healthy tubers sown in unsterilized and uninoculated soil.
C3: Untreated healthy tubers sown in sterilized soil inoculated with *Alternaria solani*.
C4: Untreated healthy tubers in sterilized uninoculated soil

Three tubers were planted per pot at a depth of 5 cm. First foliar spray was given after 21days and second after 42 days from the date of sowing. 25 ml of *Alternaria solani* suspension was sprayed per plant and the treated plants were covered with poly bags for 48h. After 48hours 30 ml of herbal formulation was sprayed per plant and left for 90 days for assessing the disease severity.[16]

The tubers were harvested after 90 days from the date of sowing and observations were recorded as the number of leaves/plant, plant height of potato, total tuber weight /pot, tuber size of potato, total number of tubers/pot. Percent disease infestation on tubers was recorded with the comparative study of positive controls. Data were subjected to analysis of CD and CV value. Three replicates were maintained with each experiment.[13,17]

**Disease Severity**

Disease severity is the measure of sickness of diseased plant. It is a qualitative trait, which measures the effect of disease on a plant tissue, intensity of symptoms or damage.[18,19]
Disease severity was calculated by using following formula:
Disease Severity = Area of tissue infected/ Total area X 100

**Growth Parameters**

Following parameters as described in Technical bulletin of Central Potato Research Institute, ICAR, Shimla was measured by standard methods in healthy, infected and treated plants:

- Number of leaves/plant
- Plant height
- Total tuber weight/pot
- Tuber size
- Number of tubers /pot

## RESULTS AND OBSERVATIONS

**Disease Severity:** Results of effect of herbal formulations on disease severity are listed in table no.1 & Fig.1. Maximum disease severity was observed with C3 (88.5%) followed by C2 (60.14%) and C4 (57.14%) respectively. As compared to this all treatments as well as mancozeb were effective in reducing the disease severity. Maximum decrease in disease severity was observed with T4 (12.5%) followed by C1 (18.75%), T3 (22.42%), T1 (30.57%), T2 (38.44%), T6 (43.24%), and T5 (47%) treatment.

Reduction in disease severity was comparable in case of C1 i.e. mancozeb and T4 i.e formulation no. 22 prepared from partially purified chloroform extract (6ml): 100% neem oil cake (2ml): 100% cow dung (2ml). Although T4 provided slightly better protection. Mancozeb proved to be better as compared to T1, T2, T3, T5 and T6. Amongst the various treatments applied reduction in disease severity was best with T4 followed by T3, T1, T2, T6 and T5 (Fig. 1). Statistical analysis at 1% and 5% CD values reveals that all the treatments are significant to reduce disease severity in field trials.

**Growth Parameters**

Results of effect of herbal formulations prepared from *Cassia fistula* fruit pulp alcoholic crude extract and partially purified chloroform fraction, powder, neem oil cake and cow dung on following growth parameters of potato crop are summarized in table no. 1 & Fig. 2 to 6.

Data clearly indicate that, treatment with herbal formulations reduces disease severity which results into significant improvement of growth of host plant.

• **Number of Leaves/plant:** Results of effect of different treatments and synthetic fungicide on number of leaves/plant are given in table no. 1 & Fig. 2. The data indicate slight reduction

in number of leaves/plant due to infection. But significant increase in number of leaves /plant was observed due to treatment with herbal formulations as well as mancozeb. Maximum increase in number of leaves/plant was observed with T4 (54.66) followed by C1 (52.66), T5 (48.33), T6 (47.33), T3 (46) and C4 (44.66) respectively. Results obtained with T1 and T2 are comparable with C3. Statistical analysis at 5% and 1% CD revealed that all the treatments are significant.

• **Plant Height:** Results of effect of different treatments on height of plant are depicted in table no. 1 & Fig. 3. Similar course of growth improvement, as observed in case of no. of leaves was recorded for plant height after treatment with herbal formulations. Treatment with formulations and mancozeb resulted in increase of plant height as compared to control. Maximum plant height observed with T4 (25.33 cm) treatment followed by T6 (24.33 cm), and T5 (23.33 cm). Amongst the treatments applied, plant height observed for T3, T1, and T2 was found to be similar to C1. At 5% and 1% CD value all the treatments were found to be significant for field trials.

• **Total Tuber Weight /Pot:** Table no. & Fig. 4 list the results of effect of treatment on average tuber weight. Significant decrease in tuber weight was observed due to infection (C2, C3 and C4). All the treatments significantly improve tuber weight as compared to maintained controls. As observed in previous cases, T4 treatment was found to be the most effective and it resulted in maximum increase in tuber weight as compared to standard i.e. C1, and other controls i.e., C2, C3 and C4. Among controls, C1 (199.44 gm) treatment was found to be better than T5 (189.21 gm) and T6 (185.5 gm). Amongst various treatments T4 (245.45 gm) was best followed by T3 (223.47 gm), T2 (210.26 gm) and T1 (201.9 gm). Statistical analysis at 5% and 1% CD revealed that all the treatments are significant.

• **Tuber Size:** Results of effect of different treatments on tuber size are given in table no. 1 & Fig. 5. Minimum tuber size was observed with C3. Slight increase in tuber size was observed with C4 followed by C2. As compared to this all treatments as well as mancozeb were effective in increasing the tuber size. Maximum increase in tuber size was observed with T4 (6.86 cm) followed by T3 (6.3 cm), T2 (5.86 cm) and T1 (5.46 cm) respectively. C1 (5.36 cm) treatment was found to be effective over T5 (5.16 cm) and T6 (4.96 cm) treatment. Statistical analysis at 5% and 1% CD revealed that all the treatments are significant.

• **Number of Tubers/Pot:** Results of effect of different treatments and synthetic fungicide on number of tubers/pot are given in table no. 1 & Fig. 6. Almost 50% reduction in tuber numbers was observed due to infection (C3 and C4). Significant improvement in number of tubers was observed with T4 treatment i.e. 18.33. T3 (16.66) was the second most effective treatment followed by T2 (14.66). Amongst the treatments applied, number of tubers/pot observed for T1 (13.66) was found to be similar to C1 and C2. C1 (13.66) and C2 (13.33) treatments were found to be effective over T5 (12.66) and T6 (10.66) treatment. Statistical analysis at 5% and 1% CD value all the treatments were found to be significant for field trials.

**Table 1: Effect of Treatments on Disease Severity and Growth parameters of Potato**

| Treatments | Percent Disease Area | Number of Leaves/Plant | Plant Height (cm) | Total Tuber Weight (gm)/Pot | Tuber Size (cm) | Number of Tubers/Pot |
|---|---|---|---|---|---|---|
| T1 | 30.57 | 44.66 | 23 | 201.9 | 5.46 | 13.66 |
| T2 | 38.44 | 41.33 | 22.66 | 210.26 | 5.86 | 14.66 |
| T3 | 22.42 | 46 | 23 | 223.47 | 6.3 | 16.66 |
| T4 | 12.5 | 54.66 | 25.33 | 245.45 | 6.86 | 18.33 |
| T5 | 47 | 48.33 | 23.33 | 189.21 | 5.16 | 12.66 |
| T6 | 43.24 | 47.33 | 24.33 | 185.5 | 4.96 | 10.66 |
| C1 | 18.75 | 52.66 | 21.66 | 199.44 | 5.36 | 13.66 |
| C2 | 60.14 | 32.33 | 20.66 | 165.88 | 4.86 | 13.33 |
| C3 | 88.5 | 43.66 | 16 | 125.16 | 3.16 | 12.66 |
| C4 | 57.14 | 44.66 | 12.66 | 155.15 | 4.23 | 9.66 |
| Mean | 41.9 | 45.6 | 21.3 | 190.1 | 5.2 | 13.6 |
| SD | 22.8 | 6.2 | 4.0 | 34.7 | 1.0 | 2.6 |
| SE m+ | 4.17 | 1.13 | 0.72 | 6.34 | 0.19 | 0.47 |
| CD (P=0.05) | *7.47 | *5.06 | *4.59 | *9.49 | *3.26 | *3.92 |
| CD (P=0.01) | *9.33 | *5.87 | *5.04 | *12.36 | *3.96 | *4.51 |
| CV (%) | 54.49 | 13.54 | 18.58 | 18.27 | 19.95 | 18.81 |

Each data represent the average of three replicates* Significance.

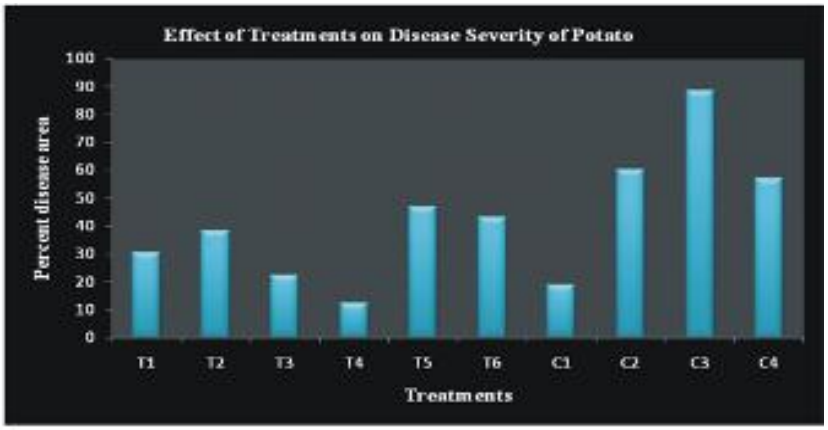


**Figure.1: Effect of Treatments on Disease Severity of Potato**

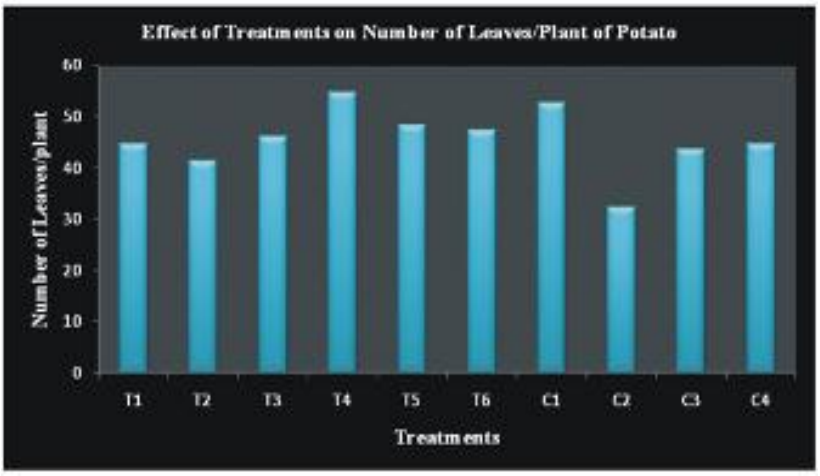


**Figure.2: Effect of Treatments on Number of Leaves/Plant of Potato**

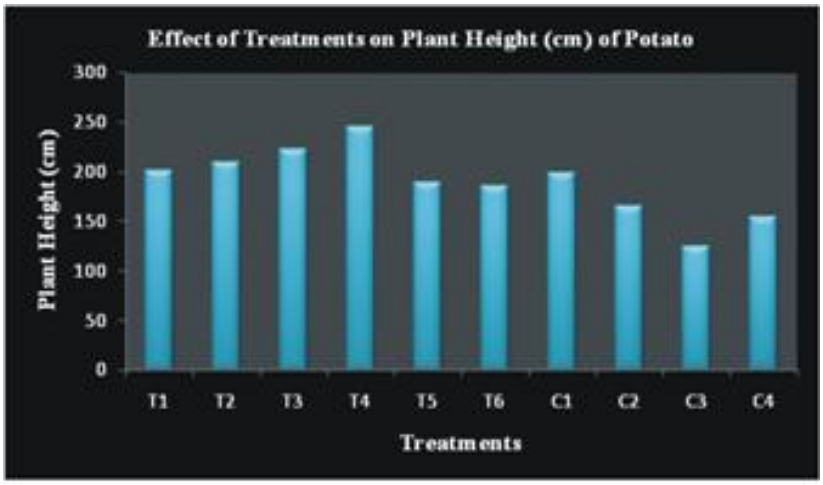


**Figure.3: Effect of Treatments on Plant Height (cm) of Potato**

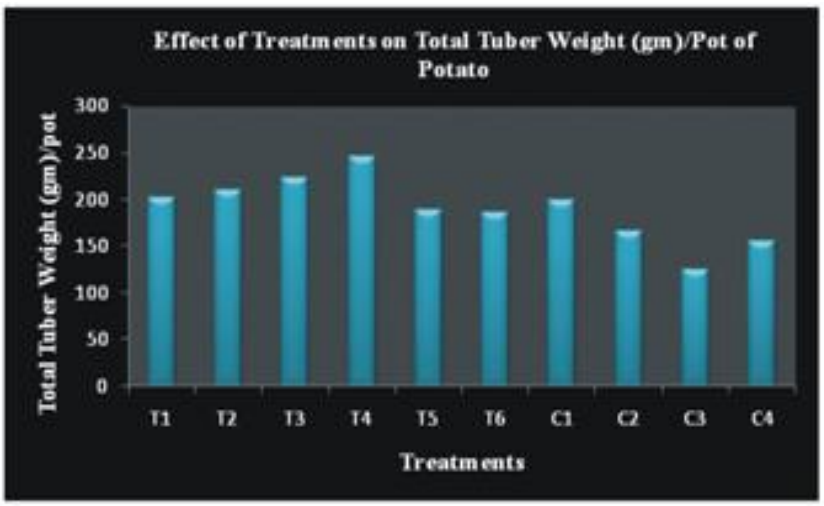


**Figure.4: Effect of Treatments on Total Tuber Weight (gm)/Pot of Potato**

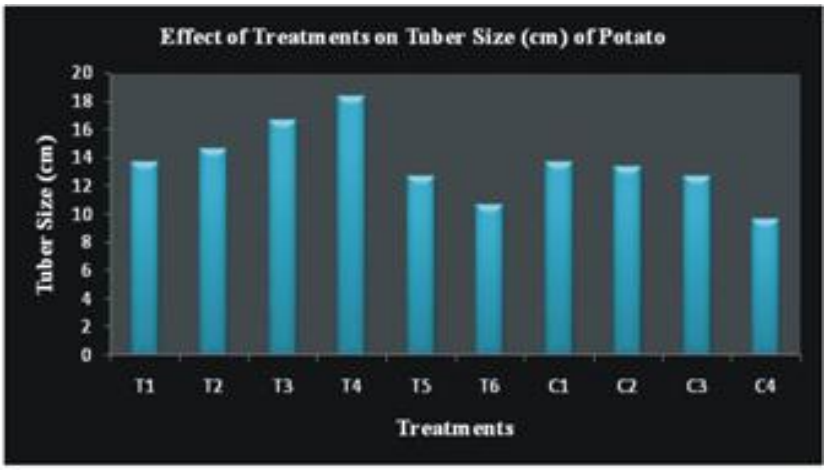


**Figure.5: Effect of Treatments on Tuber Size (cm) of Potato**

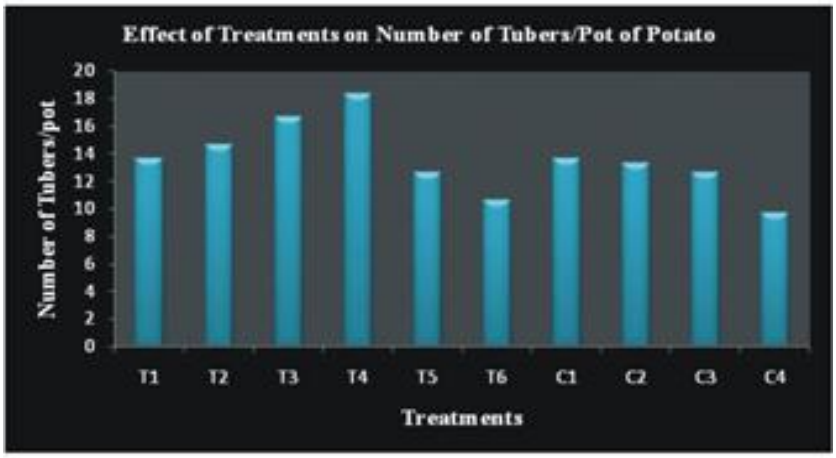


**Figure.6: Effect of Treatments on Number of Tubers/Pot of Potato**


**a** **b**

**Figure.7: (a) Healthy tubers dipped in various treatments (b) Controls (C1, C2, C3 C4) against *Alternaria solani***


**Figure.8: *In vivo* Study of Preventive effect of Herbal formulation against *Alternaria solani***

## DISCUSSION

India rank 4th in area and it is the 3rd largest country in world in production of potato after China and Russian Federation. Potato is grown almost in all states of India. In India Rajasthan state has a very negligible area under potato cultivation and restricted to limited pockets like Kota, Dholpur, Bharatpur and Alwar districts.[20] Early blight, caused by *Alternaria solani*, is a common potato (*Solanum tuberosum* L.) disease. The disease often occurs initially on older, less productive foliage, followed by a gradual upward progression within the canopy, resulting in premature leaf senescence.[21,22] If the inoculum load is high during favourable environmental conditions, early blight may become severe enough to cause significant reductions in yield.[23] Under such conditions, frequent applications of protectant fungicides are often required to reduce foliar disease severity and subsequent yield loss.

*Alternaria solani* is a very destructive fungus for potato crop, but with the utilization of advanced techniques it becomes easier to control this cosmopolitan fungus. One of the most commonly used method is the use of fungicides, but these fungicides causes serious health hazards to human beings and also they cause environmental pollution. Hence, now a days more emphasis is given on other methods of disease control like growing disease resistant varieties, alterations in agronomic practices, use of plant and natural products and herbal

formulations etc. because they are more economical, eco-friendly and safe. An effective control programme also combines cultural practices, fungicides, biological control, and solarization.[24]

Avoiding or minimizing the pesticide residues is required in the marketable products of potatoes. Therefore, one important aspect is the development of alternative control treatments based on plant extracts and herbal formulations. The fungicidal activity of some plant extracts in controlling different plant pathogens have been reported by several workers.[25,26,27] Management of plant diseases by the use of plant extracts has also been reported.[28,29]

Plants possess various inducible defense mechanisms to protect themselves against pathogen attack. Yadav *et al*. isolated compound which showed antimicrobial activity against *Staphylococcus aureus, Bacillus subtilis, Klebsiella pneumoniae, Escherichia coli, Aspergillus niger* and *Fusarium oxysporum.*[30] Ali *et al.* reported that the antibacterial and antifungal activities of *C. fistula* and *M. ferrea* extracts were tested on 14 bacteria and 6 fungi. *C. fistula* extracts showed stronger antibacterial activity than *M. ferrea*.[31] Sundararaju *et al.* reported that 100% mortality was recorded from the *C. fistula* extract at 48 h at 50 and 100% concentrations. At 72 h, 100% mortality was observed in all extracts at all three concentrations. The mortality rate was minimum at 24 h in all three extracts. All plant extracts exhibited a high degree of nematicidal action against the adults and juveniles of *P. coffeae.*[32]

Matsuzaki *et al.*, reported that soil with cow manure amendments is best treatment for reducing the severity of the disease and improving the final tubers yield of potato [33]. Kimaru *et al.*, 2004 investigate effect of Neem Kernel Cake Powder (NKCP) on development of tomato *Fusarium* wilt.[34] Hamid et al., used cow manure and soil solarisation treatment for effective suppression of potato disease caused by *R. solani* and subsequent improvement of the final tuber yield.[35]

Healthy seed tubers, treated with herbal formulation and mancozeb respectively for a comparative study, were sown in inoculated soil. Untreated tubers sown in inoculated soil served as positive control. During *in vitro* screening of synthetic fungicides mancozeb was found to be the most effective synthetic fungicide against *A. solani* hence it was used as a standard control.

Novelty of this study is the use of combination of plant extract of different purity with elicitor i.e. neem oil cake and binder i.e. cow dung, which is not yet reported in searched literature. These combinations observed to show significant activity against *A. solani* and can help to minimize the economical loss of potato crop.

A comparative study of effect of herbal formulation versus mancozeb reveals that T4 treatment i.e. formulation no. 22 was found to be significantly improved all the growth parameters as compare to other treatments. In case of number of leaves/plant and disease severity mancozeb was found to be good after T4 treatment. Results of growth parameters obtained for T1, T2, T3, T5 andT6 treatments were also significant as compare to healthy controls. Results of the comparative study between the preventive effects of herbal formulations and synthetic fungicide indicate that protection offered by treatment no. 4 was more effective over mancozeb treatment. As compared to other treatments T4 treatment significantly reduces *A. solani* infection. It may be due to the fact that the group of active metabolites responsible for antifungal activity. Preventive effect of herbal formulation on disease severity might be due to presence of secondary metabolites present in plant extract. *Cassia fistula* fruit pulp contains anthraquinone, glycosides, sennosides A & B, rhein and its glucoside, volatile oil, barbaloin, aloin, formic acid, butyric acid and their ethyl esters and oxalic acid, presence of pectin and tannin is also reported.[36]

Results of present study indicate that treatment with herbal formulation especially T4 treatment not only reduces the infection but also leads to increased growth, health and vigour of the host plant as compared to infected plant and synthetic fungicide treatment. Chakraborty and Patil, have suggested that in addition to the preventive effect natural formulations also maintain the soil health which results in healthy growth of crop.[37] Terpenes play an important role as signal compounds and growth regulators (phytohormones) of plants. Gershenzon and Dudareva, reported the role of terpenes in growth and development of plant.[38] Fungal pathogens can invade and occupy living plant cells, diverting nutrients to the growing fungus and suppressing plant defense mechanisms.[39]

## CONCLUSION

Exploitation of preparations based on natural substances, which can limit plant pathogens development comes into higher and higher prominence, especially restricting traditional chemical preparation application. It results from comparable efficiency of bio-preparations to pesticides. In the present investigation, reduction in disease severity after treatment with

herbal formulations was found to be comparable with synthetic fungicides. Hence these formulations can be used to develop eco-friendly alternatives for management of early blight diseases of potato.

Among the various treatments, T4 (Formulation no.22) treatment was found to be effective against *Alternaria solani* both during *in vitro* as well as *in vivo* trials. This treatment was not only found to be reducing the disease incidence but also helpful in improvement of all the growth parameters. However characterization of this active treatment via advanced molecular techniques like IR, NMR, GC-MS etc. and further quality assurance and then field trials could be very helpful to overcome the problem of non targeted fungicides.

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