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Eco-friendly Management of *Colletotrichum capsici* (Syd.) Butler and Bisby - The Incitant of Anthracnose of Chilli

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ABSTRACT: Aiming to develop more efficient and environmental friendly methods than existing one to manage *Colletotrichum capsici*, which causes anthracnose of chilli. The study investigate to search appropriate natural management by using some organic materials against anthracnose (*Colletotrichum capsici*) of chilli. The bioagents *viz. Trichoderma* spp, *Bacillus* spp and *Pseudomonas* spp were found effective against test pathogen. Among them, *Trichoderma hamatum* was found most effective and recorded highest percent mycelial inhibition (77.03 %) followed by *T. koningii*, (74.07 %), *T. harzianum* (67.77 %), *T. asperellum* (60.74 %) and *Bacillus subtilis* (59.63 %). The least mycelial growth of the fungus is recorded in *Pseudomonas fluorescens* (50.00 %) respectively. The plant extracts *viz.*, Neem, Tulsi, Marigold, Parthenium, Drumstick and Lantana (each @ 5 and 10%) and essential oils *viz.*, Neem, Lemon grass, Thyme, Winter green, Eucalyptus and Cinnamon oil (each @ 0.1 and 0.05 %) were evaluated against the *C. capsici*. The result revealed that, essential oils viz., Thyme oil, Eucalyptus oil, lemon grass oil (each @ 0.05 and 0.1 %) and Cinnamon oil at 0.1 % concentration was highly effective as cent percent inhibition was achieved followed by Cinnamon oil (88.51 %), neem oil (55.55 %), Winter green oil (66.66 %) with the maximum percent mycelial growth inhibition over the untreated control. The plant extract of Neem was found to be effective and showed growth inhibition percent at 10 and 5 percent conc. was 40.00 and 32.97 percent respectively,followed by Tulsi with growth inhibition percent 27.41 and 21.48 at 10 and 5 percent conc. respectively. The minimum growth inhibition was recorded in case of the Lantana with 14.08 and 21.11 percent at 5 and 10 percent conc. respectively. So, organic management might be a better option to control anthracnose of Chilli and also having environment friendly.

Keywords: Chilli anthracnose, *Colletotrichum capsici*, Ecofriendly management, Essential oils, Plant extract, Bioagent.

INTRODUCTION

Chilli (*Capsicum annuum* L.) is a globally significant crop, extensively cultivated for its dual roles as both a culinary staple and a valuable medicinal resource. India, in particular, holds a pivotal position in the global chilli market, being a major exporter, producer, and consumer of this versatile crop. Several Indian states, including Andhra Pradesh, Karnataka, Maharashtra, Odisha, and Tamil Nadu, are renowned for their substantial contributions to chilli production.

Chilli cultivation faces a myriad of challenges, primarily stemming from diseases caused by viral, fungal, bacterial, nematode and phytoplasmal diseases (Devi *et al*., 2019). Among these, anthracnose (also known as die-back) stands out as a destructive force, causing substantial losses, ranging from 10% to 60%, in both yield and fruit quality, contingent on the chilli variety. Anthracnose is pervasive in tropical and subtropical regions, afflicting countries such as India, Thailand, China, and Indonesia. In India, anthracnose is primarily caused by three species of *Colletotrichum*: *Colletotrichum capsici*, *Colletotrichum acutatum*, and *Colletotrichum gleosporoides*, with *Colletotrichum capsici* Syd. Butler and Bisby being responsible for significant fruit losses during the plant's mature stage (Saxena *et al*., 2016). This disease is particularly devastating, resulting in substantial economic losses.

Anthracnose of chilli is now an important disease found to be the major constraint in chilli production for both profitable cultivation and seed production. This devastating pathogen damages both immature and mature fruits reducing the nutritive and marketing value of chillies (Manda *et al*., 2020). Mostly the chemical fungicides were recommended for the management of this disease. Chemical disease control is very common among farmers but Eco-friendly approaches have attained importance in modern agriculture to curtail the hazards of extensive use of pesticides for managing the disease, hence, to mitigate the impact of chilli anthracnose, Trichoderma species are often employed in seed treatment and foliar sprays. Additionally, botanical solutions, including leaf extracts from Neem, Datura, Ocimum, Polyalthia, and Vinca rosea, have

demonstrated toxicity against Colletotrichum capsici (Shivapuri *et al*., 1997).

MATERIALS AND METHODS

The present experiment was conducted at Post Graduate institute, Dr. PDKV Akola (M.S.) during 2022-23. The four fungi and two bacterial bioagents was evaluated *in vitro* against *C. capsici*, by applying Dual Culture Technique (Dennis and Webster 1971). The essential oils were evaluated *in vitro* for their antifungal activities against chilli anthracnose, requisite quantity of each essential oils on the basis of active ingredient (a.i) was calculated and Tween (for oil dispersion) was thoroughly mix with autoclaved and cooled (40-45ºC) PDA in conical flasks to obtain desired concentration of 0.05 and 0.1 %. *In vitro* evaluation of six plant extracts was done at different concentrations (5% and 10%). The efficacy of plant extracts and the essential oils were tested by using Poisoned Food Technique described by Nene and Thapliyal (1993) on potato dextrose agar (PDA) medium.

Isolation and identification of *Colletotrichum capsici*. Chilli fruits having anthracnose symptoms were collected from the fields. Isolation was done by cutting small pieces from the margin of lesions which were then surface sterilized by immersing in 1 per cent Sodium Hypochlorite solution for 30 seconds and washed with sterilized distilled water. To remove excess moisture from the samples the pieces were transferred on to sterilized blotter paper. These pieces were then transferred to Petri plates containing PDA medium under aseptic conditions followed by incubation at 26±2ºC.

Purification of *Colletotrichum capsici*. The fungus was further purified by single hyphal tip method. They are grown by inoculating in the centre of a plain agar plate. The fungus spreads out with its hyphal strands in search of nutrients. These hyphal strands could be located under low power of the microscope, and the isolated hyphal tips marked. These tips were carefully transferred to potato dextrose agar slants to obtain the pure cultures of *Colletotrichum capsici*. The culture was maintained by sub-culturing on potato dextrose agar medium at room temperature.

Preparation of Botanicals solutions. To prepare the plant extract healthy and fresh leaves of selected plants were taken and washed with tap water followed by sterile distilled water and were chopped into small bits with sterilized sharp knife. Mechanical grinder was used to separately grind each sample and homogenize it with equal quantity of sterile distilled water 1:1 (w/v). The obtained homogenate was strained through double layered sterilized muslin cloth followed by filtration through Whatman's filter paper No. l. The obtained clear leaf extracts serve as the stock solution of 100 per cent and subsequently 5 and 10 per cent concentrations were made. The desired quantity of each extract was separately mixed in molten PDA medium in conical flask to get the desired concentrations (5 and 10 %).

The PDA medium amended with plant extract and essential oils were poured separately @ 20 ml per Petri plate. After solidification of poisoned medium, the plates were inoculated with 0.5mm mycelium disc of *C. capsici* obtained from seven days old culture of pathogen. Plates containing um-amended medium served as control. The inoculated plates were incubated in B.O.D incubator at 26±2˚C. The colony diameter of culture was recorded when plates under control were fully covered. The efficacy of bioagents, essential oils and plant extracts was expressed as per cent inhibition of mycelial growth over control, which was calculated by using the following formula (Vincent, 1947).

$$\text{Per cent Growth} = \frac{\text{Colony growth in Control plate} \times \text{Colony growth in treated plate}}{\text{Inhibition colony growth in control plate}} \times 100$$

RESULTS AND DISCUSSION

A. In vitro Evaluation of Bioagents

The results obtained from evaluating four fungal and two bacterial antagonists for their effects on *C. capsici* are presented in Table 1 and Plate 1 Fig. 1. These bioagents exhibited significant fungistatic and antifungal activity against *C. capsici*, effectively inhibiting its growth compared to the untreated control.

Mycelial Growth Inhibition: Regarding mycelial growth inhibition, *Trichoderma hamatum* again proved to be the most effective, achieving the highest percentage of mycelial inhibition (77.03%). It was followed by *T. koingi* (74.07%), *T. harzianum* (67.77%), *T. asperellum* (60.74%), *Bacillus subtilis* (59.63%), and *Pseudomonas fluorescens* (50.00%).

Overall, among the seven bioagents tested, *Trichoderma hamatum* and *T. koingi* were the most effective, followed by *T. harzianum*. These bioagents employ various mechanisms for control, including antibiosis, competition, superficial growth, and mycoparasitism, which involves the production of cell-wall degrading enzymes such as chitinases, cellulases, and proteases. They are saprophytic microorganisms with a comprehensive metabolism that is capable of utilizing a variety of substrates (Mukhopadhyay and Kumar 2020).

The mechanisms of control include antibiosis that is related to the production and release of several compounds to retard or inhibit the microorganism's growth (Reyes-Figueroa *et al.,* 2016). Competition and superficial growth, this mechanism is very effective when the conidia of the pathogens needed exogenous nutrients to germinate (Elad *et al*., 1981). Mycoparasitism, which is accompanied with cell-wall degrading enzymes such as chitinases (endochitinases, exochitinases, and β-*N*-acetilhexosaminidases), cellulases (exoglucanases, endoglucanases, and β-1-3-glucanases) and proteases. These enzymes are used by microorganisms to dissolve the host cell wall and then penetrate the cell to obtain nutrients (Miller, 1959).

Table 1: *In vitro* bioefficacy of different bioagents against *C. capsici*

Tr. No.	Treatments	Colony Diameter* (mm)	% inhibition
1	*Trichoderma asperellum*	35.33	60.74
2	*T. harzianum*	29.00	67.77
3	*T. hamatum*	20.67	77.03
4	*T. koingi*	23.33	74.07
5	*Bacillus subtilis*	36.33	59.63
6	*Pseudomonas fluorescens*	45.00	50.00
7	Control (untreated)	90.00	0.00
	SE±	**0.36**	-
	CD at P=0.01%	**1.47**	-

*Average of three replications

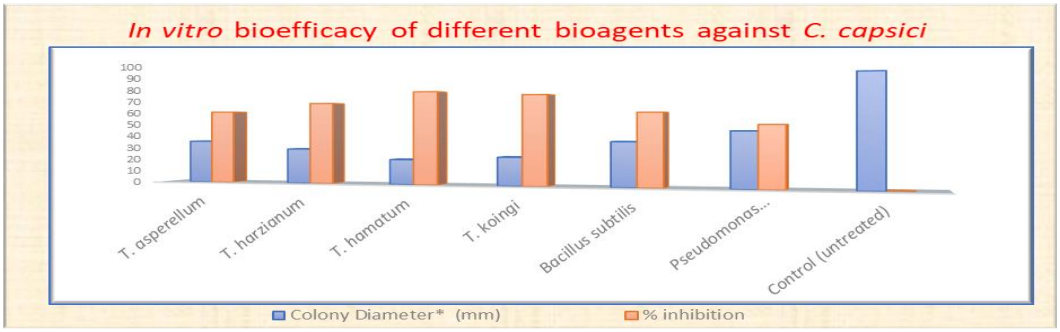

Fig. 1. *In vitro* bioefficacy of different bioagents against *C. capsici.*

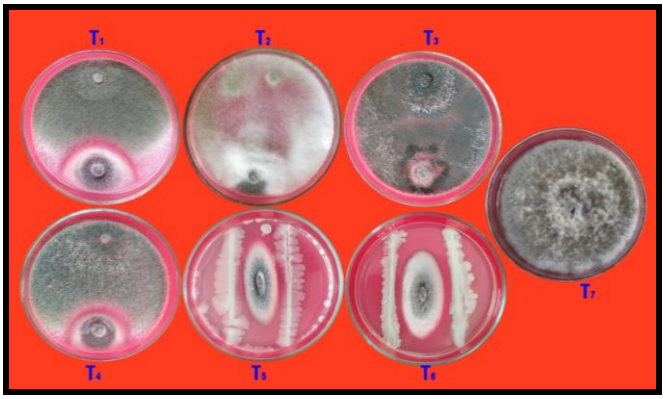

Plate 1. *In vitro* bioefficacy of different bioagents against *C. capsici.*

B. In vitro Evaluation of Essential Oils (Each @ 0.05% and 0.1%)

Six essential oils, including Neem, Lemon grass, Thyme, Winter green, Eucalyptus, and Cinnamon oil, were evaluated against *C. capsici*, as described in the materials and methods section. The data on the percentage inhibition of radial growth of the fungus are presented in Table 2.

Average Mycelial Growth Inhibition: The average radial percentage mycelial growth inhibition with the tested essential oils ranged from 55.55% to 100.00%. Thyme oil, Eucalyptus oil, and lemon grass oil (each at 0.05% and 0.1%) and Cinnamon oil at 0.1% concentration were the most effective, achieving the highest percentage of mycelial growth inhibition (100%) compared to the untreated control. Cinnamon oil at 0.05% concentration resulted in an inhibition of 88.51%. Neem oil (55.55%) and Winter green (66.66%) also exhibited significant mycelial growth inhibition compared to the control.

Table 2*: In vitro* efficacy of plant oils against *C. capsici.*

Tr. No.	Essential oils	Colony Diameter* (mm)		Mean	% inhibition		Mean
		0.05 %	0.1 %		0.05 %	0.1 %	
T_1	Neem oil	45.33	34.67	40.00	49.63	61.47	55.55
T_2	Lemon grass oil	0.00	0.00	0.00	100.00	100.00	100.00
T_3	Thyme oils	0.00	0.00	0.00	100.00	100.00	100.00
T_4	Winter green oil	35.00	25.00	30.00	61.11	72.22	66.66
T_5	Eucalyptus oil	0.00	0.00	0.00	100.00	100.00	100.00
T_6	Cinnamon oil	20.67	0.00	10.33	77.03	100.00	88.51
T_7	Control	90.00	90.00	90.00	0.00	0.00	0.00
	SE±	**0.74**	**1.02**	-	-	-	-
	CD at P=0.01%	**3.19**	**4.72**	-	-	-	-

* Average of three replications

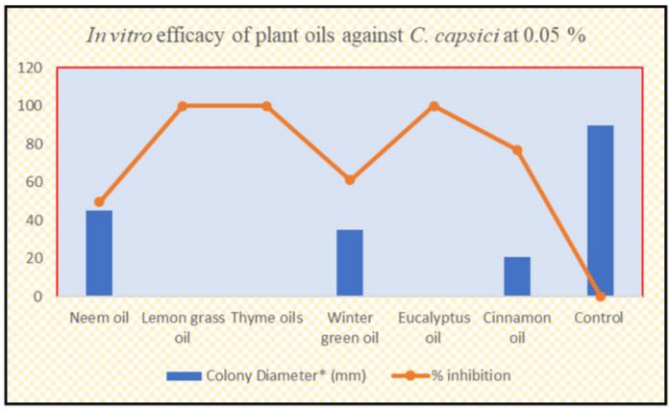

Fig. 2a. *In vitro* Efficacy of plant oils against *C. capsici.*

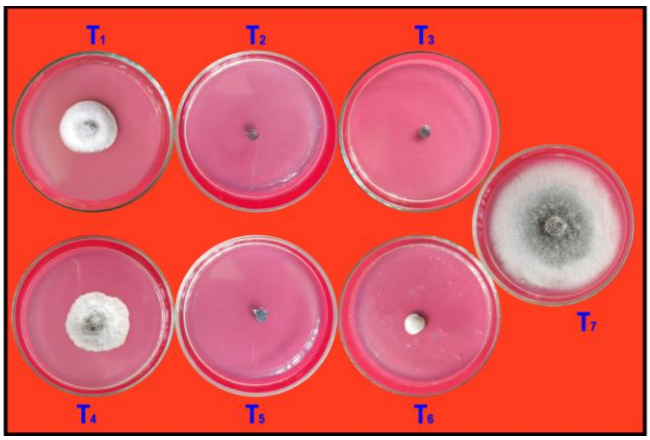

Plate 2a. *In vitro* efficacy of plant oils against *C. capsici.*

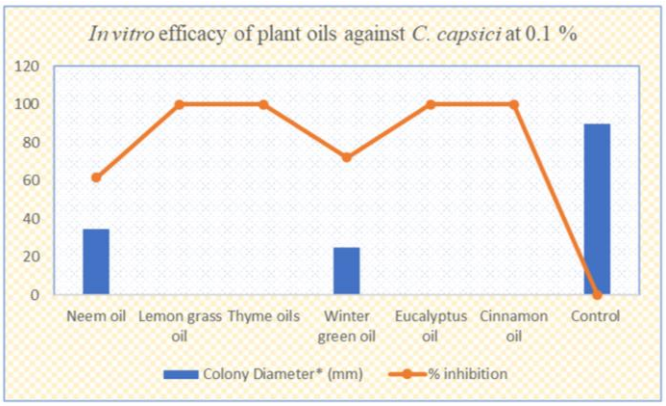

Fig. 2b. *In vitro* Efficacy of plant oils against *C. capsici.*

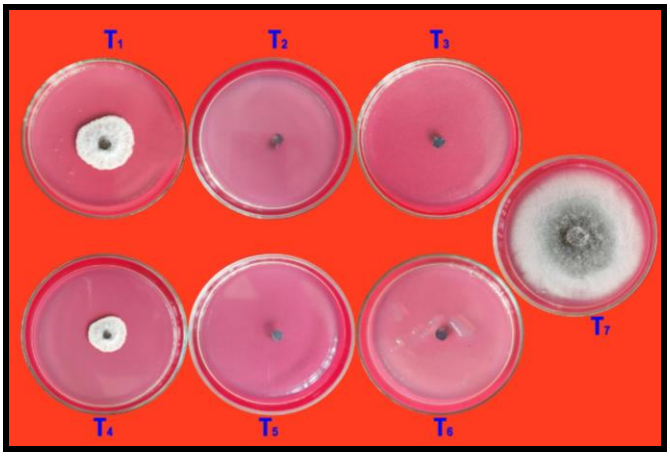

Plate 2b. *In vitro* efficacy of plant oils against *C. capsici.*

C. In vitro Evaluation of Plant Extracts (Each @ 5% and 10%)

Six plant extracts, including Neem, Tulsi, Marigold, Parthenium, Drumstick, and Lantana, were evaluated against *C. capsici*, as described in the materials and methods section. The data on the percentage inhibition of radial growth of the fungus are presented in Table 3.

Radial Mycelial Growth. Neems extract demonstrated effectiveness by inhibiting growth by 40.00% at 10% concentration and 32.97% at 5% concentration. Tulsi extract showed growth inhibition of 27.41% at 10% and 21.48% at 5%. Lantana extract exhibited the lowest growth inhibition with 14.08% at 5% and 21.11% at 10%.

Various active triterpenoids found in neem plants, such as Azadirachtin, nimbecedine, and nimbin, possess antibacterial characteristics.

Table 3*: In vitro* efficacy of plant extracts against *C. capsici.*

Tr. No.	plant extracts	Colony Diameter* (mm)		Mean	% inhibition		Mean
		5 %	10 %		5 %	10 %	
1.	Neem	60.33	54.00	57.17	32.97	40.00	36.48
2.	Tulsi	70.67	65.33	68.00	21.48	27.41	24.44
3.	Marigold	74.33	69.00	71.67	17.41	23.33	20.37
4.	Parthenium	84.00	78.67	81.34	6.67	12.59	9.63
5.	Drumstick	80.67	74.33	77.50	10.37	17.41	13.89
6.	Lantana	77.33	71.00	74.17	14.08	21.11	17.59
7.	Control	90.00	90.00	90.00	0.00	0.00	0.00
	SE±	**0.65**	**0.54**	-	-	-	-
	CD at P=0.01%	**2.85**	**2.42**	-	-	-	-

* Average of three replications

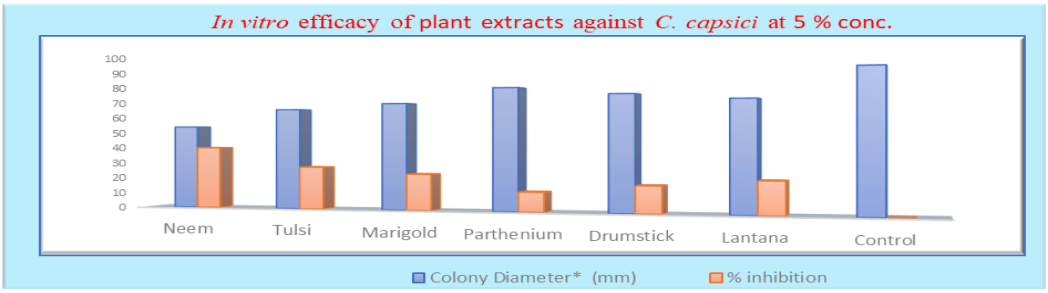

Fig. 3a. *In vitro* efficacy of plant oils against *C. capsici.*

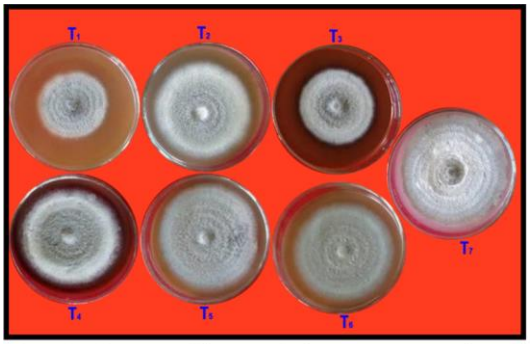

Plate 3a. *In vitro* efficacy of plant extracts against *C. capsici* at 5 % conc.

Plate 3b. *In vitro* efficacy of plant extracts against *C. capsici* at 10 % conc.

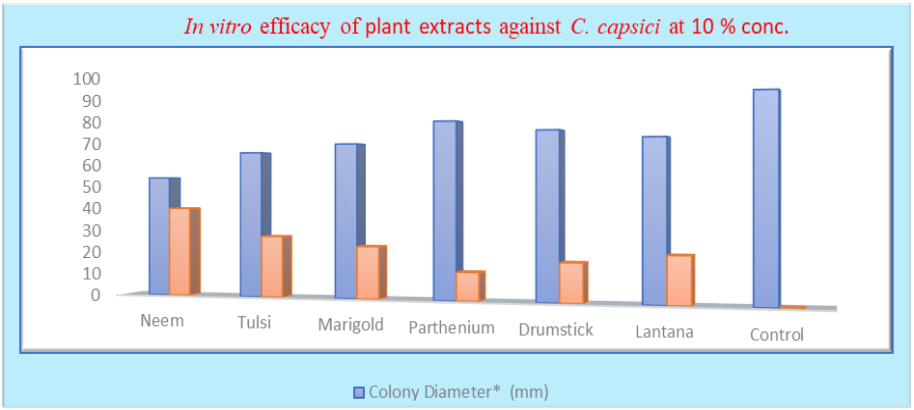

Fig. 3b. *In vitro* efficacy of plant oils against *C. capsici.*

Previous research by Kumar *et al.* (2015) showed that neem and garlic plant extracts at a concentration of 4% suppressed the mycelial growth of *C. capsici*. Similarly, Rahman *et al*. (2011) reported that extracts from *Azadiracta indica* (leaf), Ocimum sanctum (leaf), and Curcuma longa (rhizome) completely inhibited conidial germination and germ tube production of *C. capsici*. Raj *et al*. (2015) found that Tulsi at 10% concentration efficiently blocked *Colletotrichum capsici* mycelial growth at all concentrations. Similar variations in the efficacy of botanical extracts in restricting the radial growth of *C. capsici* have been demonstrated by Handiso and Alemu (2017a); Choudhary *et al*. (2017); Thaveedu *et al*. (2019); Nishanthi *et al*. (2020). These results are consistent with the findings of Ngullie *et al*. (2010), who reported that botanical extracts effectively reduced the mycelial growth of the pathogen. Begum and Nath (2015) also found that garlic, neem, and polyalthia extracts inhibited the mycelial growth of *Colletotrichum capsici* under both *in vitro* and *in vivo* conditions.

CONCLUSIONS

From present study, it was concluded that the sternness of anthracnose of chilli disease can significantly be reduced by the use of bioagents, *Trichoderma hamatum* was found most effective and recorded highest percent mycelial inhibition. The result revealed that, essential oils viz., Thyme oil, Eucalyptus oil, lemon grass oil (each @ 0.05 and 0.1 %) and Cinnamon oil at 0.1 % concentration was highly effective and also the plant extract of Neem was found to be effective against the pathogen. Recently there has been great interest in essential oils and biocontrol agents for controlling plant pathogens. Now the study shows that botanicals possess antifungal activity and can be exploited for effective management of plant diseases.

FUTURE SCOPE

— Implication of organic management measures to control anthracnose of chilli and also having environment friendly.
— To study an integrated disease management strategy for effective management of the disease.
— Cost effective methods by using the organic material available with farmers and reducing the expenditure for the management of disease.

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