Int.J.Curr.Microbiol.App.Sci (2021) *10*(05): 179-187

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 10 Number 05 (2021)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2021.1005.024

Agro Waste Amendments in the Management of *Alternaria alternata* and *Meloidogyne incognita* Diseases in Ashwagandha

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A B S T R A C T

Ashwagandha belongs to the family Solanaceae is an important medicinal plant that is used in the Indian traditional system of medicine ayurveda and unani. An experiment was conducted at the Department of Plant Pathology, SHUATS, Prayagraj and U.P. during 2018-2019 to observe Agro waste amendments in the management of soil borne diseases *i.e*. *Alternaria alternata* and *Meloidogyne incognita* of Ashwagandha. Total seven treatments (viz. Neem leaf, Leaf mold, Vegetable waste, Marigold waste, Fruits waste were taken @ 500 gm were incorporated in 10 kg of soil in the pot and Carbendazim1 kg a.i /ha were used as control. Goat manure @ 250 gm and 2 gm mycelium mat of *Alternaria alternata* per each pot were applied before incorporation of agro waste in the pots, each treatment were replicated five times. Leaf spot disease intensity caused by *Alternaria alternata* at 30, 60, 90 DAT under pot condition were recorded. Among the treatments at 30 d.a.i. the percentage disease intensity of *A.alternata* was significantly reduced in T_1-Neem (2.06), T_2-Leaf mold(3.53), T_4-Marigold waste (3.40),T_6-carbendazim(1.67)as compared with T_3-Vegetable waste (4.94), T_5- Fruit waste (4.97) and control (11.71). Where as (T_6,T_1, T_4, T_2) and (T_2,T_3,T_4, T_5)are not significantly differ from each other. At 60 d.a.i. percentage disease intensity of *A.alternata* was significantly reduced in Neem (5.82) and carbendazim (5.7) as compared to other treatments including control. However, Neem and carbendazim are not significant from one another. At 90 d.a.i. percentage disease intensity of *A.alternata* was significantly reduced in Neem (30.21) and carbendazim (26.56) as compared with T_2,T_3,T_4,$T_5$and T_0. Among the treatments (T_1, T_6), (T_1, T_4) and (T_4,T_2,T_3,T_5) are not significant from each other. The root gall population of *Meloidogyne incognita* was significantly reduced in Marigold (12.20), Neem (15.0) as compared with other treatments including control. Among the treatments (T_6, T_3, T_2) are not significant from each other but they are significantly reduced the total no. of galls from T_5 and control nematode alone.

Keywords

Alternaria alternata, *Meloidogyne incognita*, Poisoned Food Technique, Agro waste amendments

Article Info

Accepted:
12 April 2021
Available Online:
10 May 2021

Introduction

Ashwagandha (*Withania somnifera* L. Dunal) is an important medicinal plant that is used in the Indian traditional system of medicine ayurveda and unani. In India, it is widely distributed in the provinces of Madhya Pradesh, Uttar Pradesh, Punjab Gujarat and Rajasthan (Kulkarni and Dhir, 2008). The leaves are bitter and recommended in fever and tender swellings (Kaur *et al.,* 2004). The flowers are useful as astringent, depurative, diuretic and aphrodisiac (Singh *et al.,* 2011). Fruits of *Withania* have potent inhibitory effect on peroxidation of lipid (Jayaprakasam *et al.,* 2004). Leaf spot caused by *Alternaria alternata* (Fr.) Keissler is an important disease reported to be very destructive diseases which affect the growth, yield and quality of Ashwagandha (Singh 1984). The initial symptoms are leaves having brown to black spot of two to nine mm in diameter surrounded by a yellow halo appear on both dorsal and ventral surfaces of the infected leaves (Inoue and Nasu 2000). *Alternaria alternata* is the important and widely spread disease of Ashwagandha causing 50-60% yeild loss (Pati *et al.,* 2008). Compost extracts produce plant hormones; mineralize plant available nutrients, fixes nitrogen and providing useful microorganisms that colonize leaf surfaces (Zheljazkov and Warman, 2004). Mamgain *et al*.,(2013) incorporation of residues as soon as possible after harvest is another measure to reduce the harmful effects of *Alternaria*. (Pandey and kalra, 2003) reported that *W. somnifera* is highly susceptible to the root knotnematode; *Meloidogyne incognita*. Infestation results in root galling, stunted growth of the plant and low productivity. The control of plant-parasitic nematodes with organic soil amendments especially with neem based materials have proved very effective (Akhtar 1999). Gallaher (1995) found that yard-waste compost (leaves, branches, grass clippings) had little effect on either the population densities of *Pratylenchus* spp. and *Meloidogyne incognita* or the yields of a variety of vegetable crops. (Akhtar and Alam 1992) incorporation of harvested crop-residues of marigold into soil proved highly effective in suppressing the incidence of root knot caused by *Meloidogyne incognita* and in reducing the population build up of some plant parasitic nematodes.

Materials and Methods

Isolation of Fungal pathogen

The pathogen was isolated from the diseased tissues of ashwagandha by tissue segment method (Rangaswami, 1958). The infected tissues were first washed with tap water to remove the soil particles; cleaned tissues were swabbed with 80 per cent ethanol to eradicate the external microbial contaminants. The infected portions of diseased leaves were cut into small pieces using sterilized scalpel and these were surface sterilized with 0.1 per cent mercuric chloride for one minute and washed in sterile distilled water thrice and then placed on previously poured and solidified Petridish containing Potato dextrose agar (PDA) medium. These plates were incubated at room temperature (28 ± 20C) for five days and observed for the growth of the fungus. The hyphal tips of fungi grown from the pieces were transferred aseptically to PDA slants for maintenance of the culture. The pathogen was identified based on their cultural and morphological characters.

Preparation of seedlings in Nursery

It is propagated by seeds, fresh seeds are sown in well prepared nursery beds. The nursery bed were prepared by mixing with Goat manure. After 7 days of mixing goat manure ashwagandha seeds were sown by line at the depth of 1 to 3 cm. Next day light irrigation

were given, germination of the seed was started at 6-7 days after sowing, the 35 days old seedlings are transplanted in the respective pots.

Amendment of goat manure and agro waste

Before transplanting the plants of Ashwagandha Neem leaf, Leaf mold, Vegetable waste, Marigold waste, Fruits waste were taken @ 500 gm were incorporated in 10 kg of soil in the pot and Carbendazim 1 kg a.i /ha were used as control. Goat manure @ 250 gm and 2 gm mycelium mat of *Alternaria alternata* per each pot were applied before incorporation of agro waste in the pots. After the manures are incorporated in the soil. Healthy seedlings are planted to pot carefully teasing apart the tangled mass of roots and keep the pots out of direct sunlight.

Characteristics of *Alternaria alternata*

Colonies are fast growing, black to olivaceous black or greyish, and are suede-like to floccose. Microscopically, branched acropetal chains of multicellar conida (dictyoconidia) are produced sympodially from simple, sometimes branched, short or elongate conidiophores. Conidia are obclavate, obpyriform, sometimes ovoid or ellipsoidal, often with a short conical or cylindrical break, pale brown, smooth walled or verrucose. Temperature: optimum 25-28C; maximum 31-32C (Dipak *et al.*, 2013)

Collection of nematodes from infected Okra crop

Root knot infected okra plants were collected from the central field of Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagaraj. Nematode infected okra crop were collected from the field after harvesting and root galls were washed free of adhering soil particles. Infected galls were cut into small pieces and graded in electrical grinder with little amount of water.

Characteristics of *Meloidogyne incognita*

Plants affected by *Meloidogyne incognita* presents above ground symptoms of water and nutrient stress, yellowing, wiliting and stunting. Below ground galling on roots, bulbs, tubers is the typical symptom. Plant death may occur in high infestation level. The female is pear shaped about 1 mm long, with a sedentary habit, embedded in root tissues. Its pearl white body is almost completely filled by the ovaries. It does not form a cyst. The males have an ordinary worm like appearance(Jepson, 1987)

Inoculation of *Meloidogyne incognita*

One week after transplanting of seedlings in pots @10ml suspension was inoculated in each pot with beaker near the root zone.

Disease intensity

Per cent disease intensity was recorded at 30, 60 and 90 days after incidence of *Alternaria alternata* according to method (Singh, 1988).

$$\text{Disease intensity (\%)} = \frac{\text{Sum of all disease ratings}}{\text{Total number of leaves/plant} \times \text{Maximum disease grade}} \times 100$$

The data presented in table 1.1 and depicted in figure 1.1 reveals the response of effect of agro waste amendments under pot condition are significantly reduce the disease incidence of *Alternaria alternata* at 30, 60, 90 DAI under pot condition. Among the treatments at 30 d.a.i. the percentage disease intensity of *A.alternata* was significantly reduced in Neem (2.06), Leaf mold (3.53), Marigold waste (3.40), carbendazim (1.67) as compared with

Vegetable waste (4.94), Fruit waste (4.97) and control (11.71). Where as (T_6, T_1, T_4, T_2) and (T_2,T_3,T_4, T_5) are not significantly differ from each other. At 60 d.a.i. percentage disease intensity of *A.alternata* was significantly reduced in Neem (5.82) and carbendazim (5.7) as compared to other treatments including control. However, Neem and carbendazim are not significant from one another. At 90 d.a.i. percentage disease intensity of *A.alternata* was significantly reduced in Neem (30.21) and carbendazim (26.56) as compared with T_2, T_3, T_4, T_5 and T_0. Among the treatments (T_1, T_6), (T_1, T_4) and (T_4, T_2, T_3, T_5) are not significant from each other. (Pati *et al.,* 2008) reported that leaf spot disease caused by *Alternaria alternata* is the most prevalent disease of Ashwagandha. Mamgain *et al.*,(2013) incorporation of residues as soon as possible after harvest is another measure to reduce the harmful effects of *Alternaria*.

Similarly findings have been reported by Sana *et al.,* (2015) revealed that among the soil amendments, neem was found to be best in reducing disease intensity of the pathogen when compared with other treatments.

Results and Discussion

Results (1.2) revealed that the tested G.M with Agro waste amendments of Neem, Leaf mold, Vegetable waste, Marigold waste, Fruit waste and Carbendazim proved in reduction the population of *Meloidogyne incognita* in ashwagandha roots over control.

The data presented in table 1.2 and depicted in figure 1.2 reveals the response of effect of Goat manure with agro waste amendments on root gall population of *Meloidogyne incognita* of Ashwagandha at 120 DAS under pot condition. The results of 120 DAS indicate that all the treatments are significantly reduce the root gall population compared with control. The treatments were T_4 - Marigold - (12.20) followed by T_1 - Neem - (15.00), T_6 - Carbendazim -(25.00), T_2 - Leafmold -(26.80), T_3 -Vegetable waste - (27.80), T_5- Fruit waste - (54.60) are not significantly differ the root galls population of *Meloidogyne incognita* in the roots of Ashwagandha. Fruit wastes are significantly reduced from T_0 - (Nematode alone - 68.00). The treatments (T_4, T_1) (T_1, T_6) (T_6, T_2, T_3) (T_2, T_3) and (T_5, T_0) are non significant from each other.

Maximum percentage of reduction over control was obtained is T_4 -Marigold (82.05) and T_1 - Neem (77.94) followed by T_6 - Carbendazim (63.23), T_2 - Leaf mold (60.58), T_3 - Vegetable waste (59.11) and T_5 - Fruit waste (19.70).

Pandey and kalra, (2003) reported that *W. somnifera* is highly susceptible to the root knotnematode; *Meloidogyne incognita*. Infestation results in root galling, stunted growth of the plant and low productivity. The control of plant-parasitic nematodes with organic soil amendments especially with neem based materials have proved very effective (Akhtar 1999). Gallaher (1995) found that yard-waste compost (leaves, branches, grass clippings) had little effect on either the population densities of *Pratylenchus* spp. and *Meloidogyne incognita* or the yields of a variety of vegetable crops.

Similarly findings have been reported by (Akhtar and Alam 1992) Incorporation of harvested crop-residues of marigold, mustard and sunflower into soil marigold was proved maximum reduction in intensity of nematode in development of root galls caused by *Meloidogyne incognita* in all the amendment treatments and in reducing the population build up of some plant parasitic nematodes.

Table.1 Effect of goat manure with agro waste amendments on percentage of disease intensity caused by *Alternaria alternata* at different day's of intervals.

S.No	Treatments	30 DAT	%Inhibition	60 DAT	%Inhibition	90 DAT	%Inhibition	Mean
T_0	Control	11.71		31.21		79.00		40.64
T_1	G.M+Neem	2.06	82.40	5.82	81.35	30.21	61.75	12.68
T_2	G.M+Leafmold	3.53	69.85	12.05	61.39	37.79	52.16	17.79
T_3	G.M+Vegetablewaste	4.94	57.81	12.47	60.04	37.95	51.96	18.45
T_4	G.M+Marigoldwaste	3.40	70.96	11.14	64.30	35.11	55.55	16.55
T_5	G.M+Fruitwaste	4.97	57.55	16.07	48.51	40.58	48.63	20.54
T_6	G.M+Carbendazim	1.90	83.77	5.77	81.51	26.56	66.37	11.43
F- test		S		S		S		
S. Ed. (±)		0.788		1.916		2.504		
C. D. (P = 0.05)		1.670		4.062		5.309		

Table.2 Effect of Goat manure with agro waste amendments on root gall population of *Meloidogyne incognita* of Ashwagandha.

S.No	Treatments	Root-gall population after 120 DAS oldplants of Ashwagandha	
		Mean	% reduction over control
T_0	Control (Nematode)	68.00	
T_1	G.M+Neem+Nematode	15.00	77.94
T_2	G.M+Leaf mold+Nematode	26.80	60.58
T_3	G.M+Vegetable waste+Nematode	27.80	59.11
T_4	G.M+Marigold waste+Nematode	12.20	82.05
T_5	G.M+Fruit waste+Nematode	54.60	19.70
T_6	G.M+Carbendazim+Nematode	25.00	63.23
S. Ed. (±)		5.990	
C. D. (P = 0.05)		12.699	

Fig.1 Diseased symptoms on leaves

Fig.2 Effect of goat manure with agro waste amendments on percentage of disease intensity caused by *Alternaria alternata* at different day's of intervals.

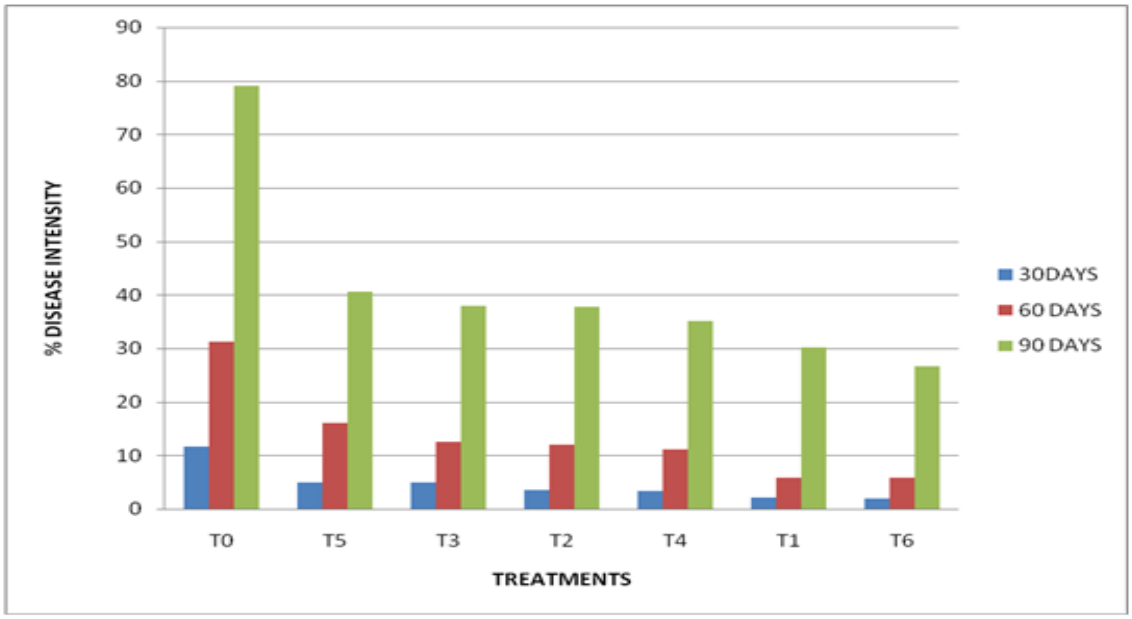

Fig.3 Effect of Goat manure with agro waste amendments on root gall population of *Meloidogyne incognita* of Ashwagandha

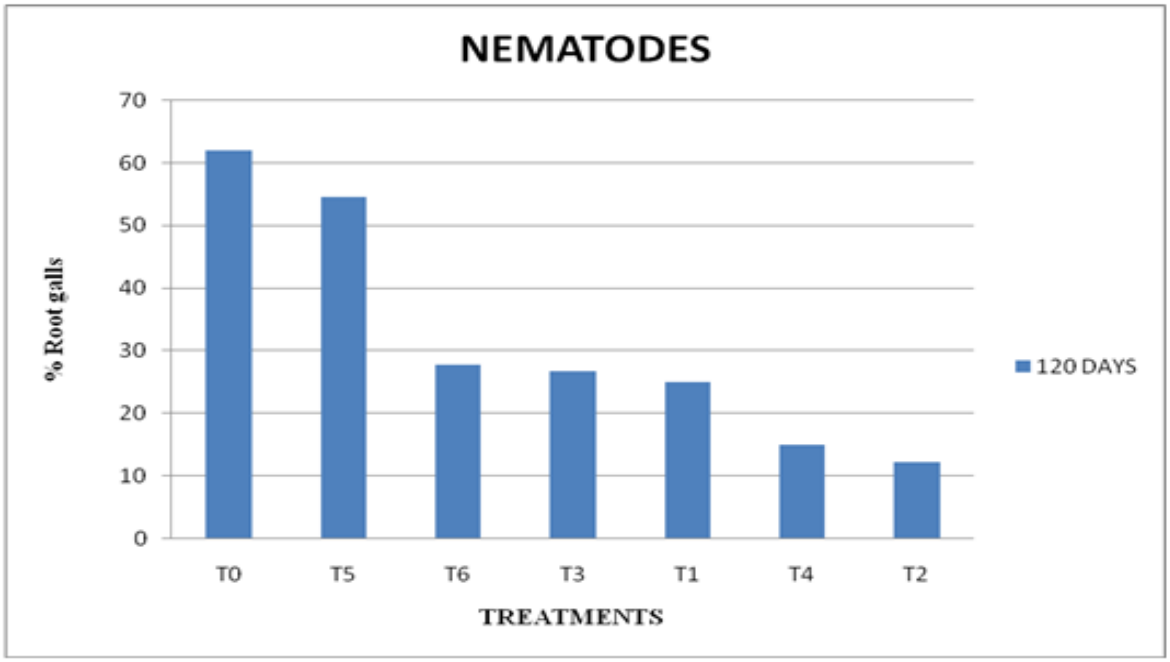

Fig.4 A. Control (Nematode alone), B. Neem+Goat manure + Nematode, C. Leafmold+Goatmanure+Nematode, D. Vegetable waste+Goatmanure+ Nematode, E. Marigoldwaste+Goatmanure+Nematode, F. Fruit waste+Goatmanure+ Nematode, G. Carbendazim+Goatmanure+Nematode

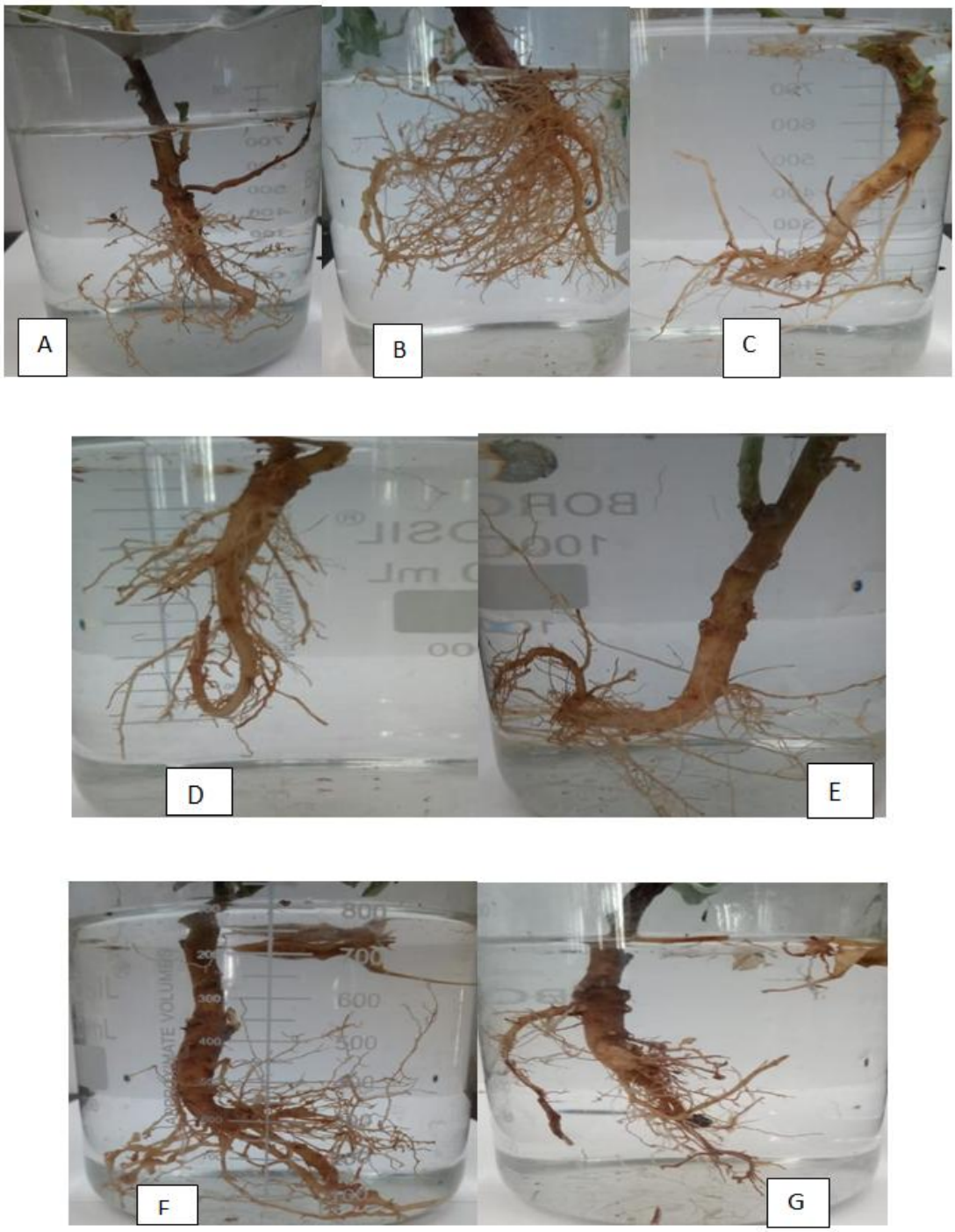

Plate.1 Effect of goat manure with agro waste amendments on *Alternaria alternata* and *Meloidogyne incognita*

Based on the results it is concluded that Neem and Marigold extract amended soil was proved to be most effective against disease intensity of *Alternaria alternata*, Marigold and Neem were proved to be most effective against root-gall population of *Meloidogyne incognita*

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How to cite this article:

Mullangi Pranitha, Pillakathupu Gopi Krishna and Sobita Simon. 2021. Agro Waste Amendments in the Management of *Alternaria alternata* and *Meloidogyne incognita* Diseases in Ashwagandha. *Int.J.Curr.Microbiol.App.Sci.* 10(05): 179-187.
doi: https://doi.org/10.20546/ijcmas.2021.1005.024