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Ramesh Kumar
Department of Plant Pathology, School of Agriculture, Lovely Professional University, Phagwara, Punjab, India

Mohammed Faraz Ahmed
Department of Plant Pathology, School of Agriculture, Lovely Professional University, Phagwara, Punjab, India

Poonam Pandurang Shete
Department of Plant Pathology, School of Agriculture, Lovely Professional University, Phagwara, Punjab, India

Corresponding Author:
Ramesh Kumar
Department of Plant Pathology, School of Agriculture, Lovely Professional University, Phagwara, Punjab, India

To study *in vitro* evaluation of selected botanicals and bioagent on *Alternaria leaf blight* of mustard caused by *Alternaria brassicae* (spp.)

Ramesh Kumar, Mohammed Faraz Ahmed and Poonam Pandurang Shete

Abstract
Oilseed Brassica spp. is found to be one of the most important diseases of oilseed crop in the world. It is susceptible to a number of diseases which is generally caused by the living pathogen. The most destructive disease in the world is found to be *Alternaria* black spot diseases which is surrounded with yellow colours. Character of the diseases is generally known by the different names which are going to be follows, *Alternaria brassica*, *Alternaria brassicae*, and *Alternaria raphani.* Alternaria leaf spot *disease* cause reduction in defoliation which produce lesion around stem, leaves and silique. These types of pathogens are found to be the air borne, seed borne and soil borne diseases. The leaf spot of diseases is generally caused by the heavy rainfall and the weather with the more disease's incidence. The severity of the diseases is also responsible for Conidial age of the host plant. This disease is more prominent during the summer seasons where the temperature falls 27- 28 °C. This paper also determines the development of *Alternaria leaf blight* in Mustard crop in relation to the pathogen such as taxonomy, biology, epidemiology, and their management through, biological, chemical, cultural and botanical approaches. *Alternaria leaf blight of mustard* plant represents the future outlook and well qualified strategy.

Keywords: *Trichoderma harzianum*, *Trichoderma viridae*, Neem, Tulsi and Ginger

Introduction
Indian mustard (*Brassica juncea* (L) Czern and Coss.) is one of the most important oilseeds crops. Which contribute around 80 percent of total rapeseed - mustard produced in India (AICRPRM, 2011) [1]. It is considered to be one of the largest produced oilseeds in the world having an area of 37.0 m/ ha, with their production of 63.09 m tonnes and their productivity of 18.50q/ha. (Singh *et al*. 2014) [17].

In India it has the area of 6.3 m hac with the production of 7.6 m tonnes and productivity of 11.90 q/hac. India contributes 28.3% and 19.8% in world acerage and production. In Punjab, the area and production under these crops were 27 thousand hectare and 33 thousand tonnes, respectively during 2008-09 (Anonymous 2010) [3]. Indian mustard is cultivated in the states of Assam, Bihar Gujarat, Haryana, Himachal Pradesh, Uttar Pradesh, Punjab, Orissa, Madhya Pradesh, Jammu and Kashmir, and West Bengal as a Rabi crop.

Alternaria leaf blight caused by *Alternaria species* is one of the major diseases of mustard (Meena *et al*, 2016, Selvamani *et al*, 2014 Jha *et al*, 2013, Aneja *et al*, 2013, Fakir 2008, Verma *et al*, 1994, Degenhardt *et al*.1974) [10, 13, 5, 16]. This disease reduces mustard yields up to 47% in India (Sharma, 2009) [18]. It is an economically important pathogen in the western Canada (Bansal *et al*, 1990) [10], in several countries of Europe and Southeast Asia. (Conn, 1990) [15]. It is a prominent disease in India, Australia, Canada, Africa, England, Germany, Sri Lanka, France, Spain, and Sweden. (Ghasemi *et al*, 2013) [22].

The crop can tolerate a wide range of soil pH, and can be grown in light and sandy to marginal soils, though rich soils particularly light loam to heavy loams, are best suited for their growth. The crop has been relatively adopted to cool and the moist climate during growing seasons and drying climate at harvesting.

Seeds of mustards/rapeseed mustards are known by the different names in different places e.g., sarson, rai or Raya, toria or lahi. While toria and Sarson are collectively termed as "rapeseed" and rai or Raya or lahi as "mustard". The oil content varies from 30 to 40 percent depending upon type and varieties of mustard or rapeseed. The mustard seeds and their oil are used in the preparation of pickles and also used for flavouring vegetables and curries.

Mustard oil is used for human consumption throughout Northern India for cooking and frying purposes. In tanning industry, mustards oil is used for leather softening. It is also used in the hair oil and medicine, in soap making, and with mineral oil with lubrication. The oil seed cake is used as cattle feed and manure. Green stems and leaves are good source of fodder for cattle.

Mustard crop is affected by the several diseases caused by bacteria, fungi, virus and nematodes. Major fungal diseases of mustard are *Alternaria blight*/ black spot (*Alternaria brassicae*, *Alternaria brassicola*, *Alternaria* raphanin), Anthracnose *(*Colletotrichum gloeosporoides), Black leg (*Leptosphaeria maculans*), Cercospora leaf spots (*Cercospora brassicola*), etc. Among these diseases, *Alternaria blight* incited by *Alternaria brassicae* (Berk) Sacc. Is an economically important and it is widely distributed diseases throughout the world on mustards and other cruciferous crops.

The diseases appear as brown or greyish spots on the leaves, stem, and on siliquae during ripening stages. *Alternaria blight* causes substantial's yield losses as a result of several factor including reduced photosynthesis potentials, Early defoliation, flower bud abortion, premature ripening siliquae, dehiscence, seed shrivelling, reduced seed sizes, and impairs seed colours and epidemiology, host pathogen interaction, and its management through various approaches.

The yield losses in the range of 35-60 percent due to *Alternaria* blight in mustard leaves were reported from India (Kadian and Saharan, 1983, Kolte 1987, Tripathi 1987, Ram and Chauhan, 1998 and Kumar, 1998) [19, 8, 15, 12].

Spores are produced in the chains or in branching fashions which are multicellular pigmented. The spores are broadest near the base and elongate beak taper gradually. The initial site of host leaf *Alternaria* produces a series of concentric rings. (Anju *et al*, 2013) [1]. The pathogen infects all aerial plant parts, reducing photosynthetic area, and accelerating senescence and defoliation. *Alternaria species* can affects all stages of the plant growth, including seed. At the seedling stage the diseases are characterized by the dark stem lesions just after germination that leads damping –off, or stunted seedlings. These types of symptoms may be varying with the host and the environment. These spots may coalesce resulting complete blackening of siliquae. Symptoms are appearing on the lower leaves with appearance of black point at first, at later the point enlarge to develop into prominent, round, concentric, spots of the various sizes.

Alternaria species is a necrotrophic pathogen produce lesion on leaves, stem, and siliquae which affect seed quality, quantity, by reducing the oil content, seed sizes and the seed colours (Duczek *et al*, 1999) [18]. This disease may cause a significant loss in both temperate and the tropical brassica crops. (Math pal *et al*, 2011) [9] The pathogen remains viable in the diseased plant debris and the seed of infected plants (Ansari *et al*, 1989) [4].

The spores of *Alternaria brassicae* can remain viable as long as 20 months in seed coats or as mycelium in infected leaf samples when it is stored at 0^{o} c and can remain viable and pathogenic up to six months when it is exposed to outdoor weather conditions at a temperature ranging between -23 to 30^{o} c. Under favourable condition symptoms will appear as defoliation of leaves on the lower leaves near ground surface which gradually progresses upwards towards the middle and the upper leaves.

Though this disease can be managed to some extent, by the use of fungicides (Verma and Saharan 1994) [16] but the environmental imbalance is the major problem in the various regions due to the injurious application of the fungicides. The antimicrobial property of some plant extracts under *in vitro* and have been reported (Mehta and Mehta 2005 Kumar *et al*, 2006) [23, 22]. A number of plant species have been found to possess some natural substances in their leaves and bulbs which were toxic to many fungi causing the plant diseases (Singh *et al*, 1991) [21].

Severe infection caused by diseases creates a substantial's yield loss as result of early defoliation, flower bud absorption, pre-mature ripening, siliqua dehiscence and seed shrivelling (Seidle *et al*, 1995) [14]. But it is reported that in India, the yield losses due to this disease of 15-71% were reported (Kumar 1997, Ram and Chauhan 1998) [6, 12].

To overcome some of these problems, the present investigation must be taken carefully to study the effect of plant extracts, like neem and the garlic paste and *in vitro* we use *Trichoderma harzianum* and *Trichoderma viridae* the weather factors on severity of the diseases to understand carefully diseases management strategy of *Alternaria blight* diseases.

The incidence of the diseases is influenced by a number of factors that include weather parameters, etc. The control measures of this diseases have been attempted through some plant extract like neem and garlic paste and other *in vitro* condition we use *Trichoderma harzianum* and *Trichoderma viridae* means, under the changing weather condition will made to investigate the effective control of the diseases.

Materials and Methods

The current research was carried out in the month of November 2020 on Lovely Professional University, Phagwara, Punjab. In-vitro evaluation will be done by using the Poisoned Food Technique (PFT) in which 1 mm mycelial discod pure culture pathogen will be taken in sterile petri-plates containing autoclaved and cooled PDA medium and different concentrates of the bioagents and extract taken for study will be incorporated along with PDA alone. Three replications for each of the concentration will be maintained along with controls and incubated inside the BOD incubator at about 25 ^{o}c for about 7 days until the mycelial growth covers the petri-plates in the control treatments. The growth on each concentration will be measured and the data will be pooled for each replication and efficacy will be studied. Least concentration of a bioagents. Or extract showing maximum restriction to the growth and spread of the fungal mycelium will be considered most efficacious.

The various bioagents and bio-extract will be taken care of for the Poisoned Food Technique (PFT) like Zinger, Tulsi and neem extract and bioagents like *Trichoderma harzianum* and *Trichoderma viridae* for Food Poisoned Technique purpose. Plant species reported to exhibits antifungal and therapeutic properties against fungal pathogen and available locally were collected from the farms of Lovely Professional University, College of Agriculture and adjoining fields. Following locally available botanical species like Neem (Azadirachta indica), Ginger and Tulsi were generally used for *in vitro* studies. For *in vitro* studies, pure culture of biocontrol agents like *Trichoderma harzianum* and the *Trichoderma viridae* were obtained from the department of plant pathology, College of Agriculture, Lovely Professional University. Maintained and multiplied on appropriate culture media for further studies.

Two fungal antagonists *Trichoderma harzianum*, and the Trichoderma viridae were evaluated *in vitro* again t *A.*

brassicae, applying dual culture technique (Dennis and Webster, 1971) [4]. Seven days old cultures of the test bioagents and test fungus *(A. brassicae)* grown on agar media were used for the study. Culture growth of the test fungus and the bioagent were cut out with the well sterilized corkborers with a Disc of sizes (5 mm dia).Then the test fungus and bioagent were going to be placed aseptically with the help of two culture discs at equidistance and opposite with each other on solidified PDA medium in Petri plates and these plates were placed on the PDA plates inoculated only with culture disc and incubated at 24+ 1°c of the test fungus were going to be maintained as untreated control.

Aqueous leaf extract of the test botanicals was prepared by grinding with mixture-cum grinder. The100 gm washed leaves of Neem, Ginger and Tulsi were macerated in 100 ml distilled water separately and macerate was filtered through double layered muslin cloth. Each of the filtrates obtained were further filtered through Whatman No 1 filter paper by using funnel and volumetric flask (100 ml cap). The final clear extract/ filtrates obtained formed the standard plant extracts of 100 percent concentration. These were evaluated (@10% and 20% each) *in vitro* against *Alternaria brassicae*, applying poisoned food technique (Nene and Thapliyal, 1993) [11] and using Potato Dextrose Agar (PDA) as basic culture medium.

An appropriate quantity of each plant extract (100%) was separately mixed thoroughly with PDA medium in conical flask (250ml cap) to obtain desired concentration of 10 and 20 percent and autoclaved at 15Ibs/inch square pressure for 15 and 20 minutes. Sterilized and cooled PDA medium amended separately with plant extract and then poured (15 to 20 ml per plate and plant extract replication) into sterile glass petri-plates (90 mm dia) and allowed to solidify into room temperature. Each plant extract and its respective concentration were replicated thrice. Untreated control was maintained as the plates containing plain PDA without any plant extract. Upon solidification of PDA, all the treatment and control plates were aseptically inoculated by placing in the centre a 5 mm mycelial disc obtained from a week actively growing pure culture of *Alternaria brassicae*. Plates containing plain PDA and inoculated with mycelial disc of the test fungus serves as untreated control. All these plates were then incubated at 24+1°c temperature for a week or till the untreated control plates were fully covered with mycelial growth of the test fungus.

Results and Discussions

Aqueous leaf extract of the test botanicals was prepared by grinding with mixture-cum grinder. The100 gm washed leaves of *Neem*, *Ginger* and *Tulsi* were macerated in 100 ml distilled water separately and macerate was filtered through double layered muslin cloth. Each of the filtrates obtained were further filtered through Whatman No 1 filter paper by using funnel and volumetric flask (100 ml cap). The final clear extract/ filtrates obtained formed the standard plant extracts of 100 percent concentration. These were evaluated (@ 10% and 20% each) *in vitro* against *Alternaria brassicae*, applying poisoned food technique (Nene and Thapliyal, 1993) [11] and using Potato Dextrose Agar (PDA) as basic culture medium.

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Table 1: *In vitro* effect of different plant extracts on growth and Inhibition of different Plant extract

Treatments	Colony Diameter (mm) at Conc.		Mean (mm)	% Inhibition at conc.		Mean% Inhibition
	10%	20%		10%	20%	
Ginger	34.16	36	35.08	43.34	40.29	41.79
Neem	36	34.83	35.41	40.29	42.23	41.26
Tulsi	36.83	33.66	35.24	38.92	44.17	41.54
Control	60.3			(00.00)	(00.00)	(00.00)

Radial Mycelial Growth

Results (Table 1) indicate that all the botanicals/ plant extracts tested exhibited a varied range of radial mycelial growth of the test pathogen. Depending upon their concentration it was ranged in increased or decreased in botanicals tested.

At 10%, radial mycelial growth of the test pathogen ranged from 36 mm. (*A. Indica*), whereas, Ginger ranged from 34.16mm and finally the mycelial growth of the test pathogen of Tulsi followed 36.83 mm over untreated control at 60.3 mm.

At 20%, the radial mycelial growth of the test pathogen ranged from 34.83 mm. (*A. Indica*), whereas Ginger ranged from 36 mm and finally the mycelial growth of the test pathogen of Tulsi followed 33.66 mm over untreated control 60.03 mm.

The Mean radial Mycelial growth recorded with the plant extracts tested (@ 10% and 20%) each was ranged from 35.41mm (*A. Indica*), whereas Ginger ranged from 35.08 mm and the Tulsi ranged followed is 35.24 mm.

Mycelial growth Inhibition

Results (Table 1) revealed that all the plant extract tested, significantly inhibited the mycelial growth of the test fungus over untreated control (00.00%). At 10% percent, percentage mycelial growth was recorded with Neem 40.29% whereas, Ginger followed the mycelial growth 43.34% and the Tulsi Mycelial growth ranged from 38.92%.

At 20% percent, percentage mycelial growth was recorded with Neem 42.23% whereas, Ginger followed the mycelial growth 40.29% and the Tulsi Mycelial growth ranged from 44.17%.

Mean percentage mycelial growth inhibition recorded with all the botanicals tested was ranged from 41.79%. However, Ginger was found most fungi static and it is recorded with highest mean mycelial growth inhibition 41.79%. And the second-best plant extract was found to be in the range of 41.54% Tulsi, and the lowest mycelial growth was recorded with neem that is 41.26%.

Thus, all the plant extract tested were found to be the fungi static/and antifungal against *Alternaria brassicae* and significantly inhibited its Mycelial growth over untreated control. Finally, Ginger was found to be the highest Mycelial growth that 41.79% followed by Tulsi and Neem.

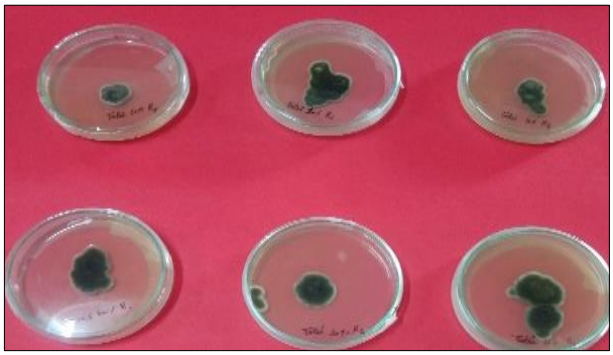

Fig 1: Tulsi

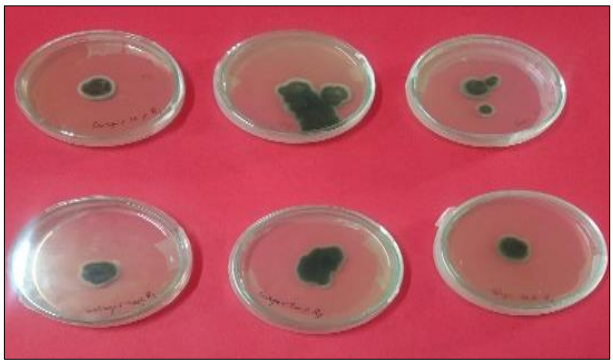

Fig 2: Ginger

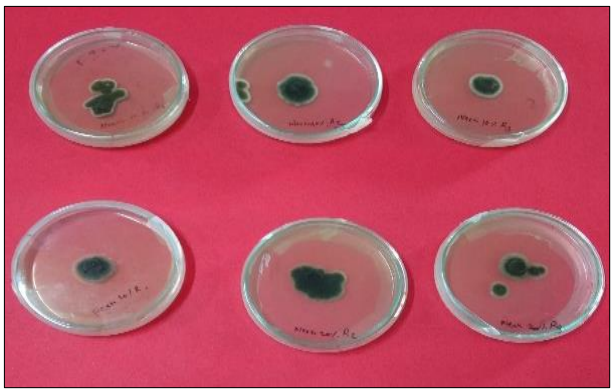

Fig 3: (Neem): *In vitro* effect of Plant extract at 10% and 20% on growth and Inhibition of *A. brassicae (*after 3 days)

Fig 4: Tulsi

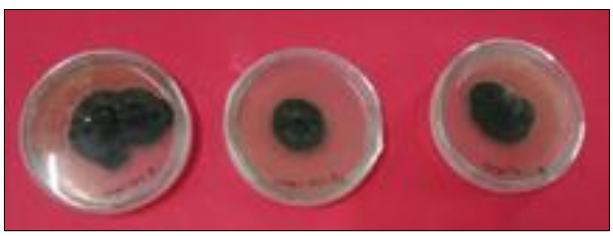

Fig 5: Ginger

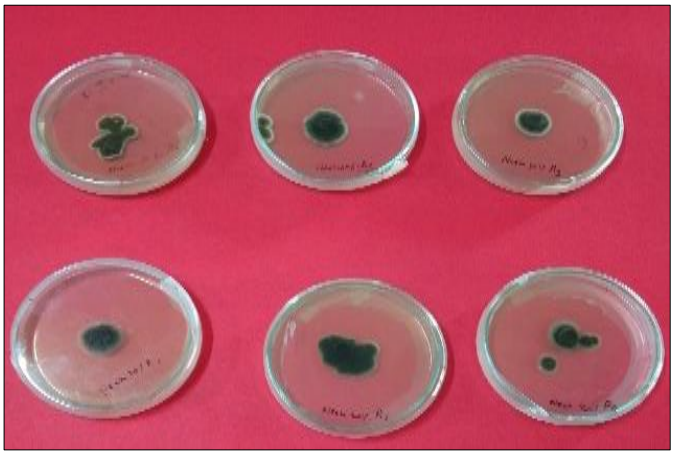

Fig 6: Neem

Fig 7: Control *In vitro* effect of Plant extract at 10% and 20% on growth and Inhibition of *A. brassicae (*after 7 days)

Two fungal namely as *(Trichoderma harzianum and Trichoderma viridae)* bioagents were evaluated *in vitro* against *Alternaria brassicae* by applying dual culture technique (Dennis and Webster, 1971) [4] and using PDA as basal medium.

Table 2: *In vitro* effect of different bioagents on growth and Inhibition of *A. brassicae*

Bio controls	Colony diameter (mm)	% Inhibition
T. harzianum	20.5	15.14
T. viride	19.16	20.69
Control	24.16	

Results (Table 2) revealed that all the bioagents exhibited fungi static activity and significantly inhibited mycelial growth of *Alternaria brassicae* over untreated control. Of the two bioagents tested, *Trichoderma viridae* was found to be the most effective which recorded significantly least linear mycelial growth that is (19.16mm) and corresponding highest mycelial growth inhibition (20.69%) of the test pathogen over untreated control that is (24.16). and the second and the third best bioagents that is *Trichoderma harzianum* which is recorded highest Mycelial growth that is (20.5 mm) and corresponding Mycelial growth inhibition that is (15.14%) of the test pathogen of the untreated control over (24.16 mm).

Thus, all the fungal bio Fig (6) Neem

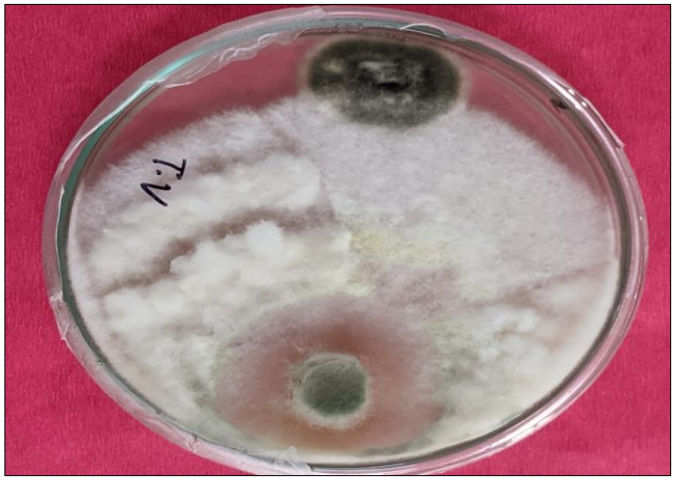

Fig 8: *In vitro* effect of bioagents on growth and inhibition of *Alternaria brassicae* (*Trichoderma viridae.*)

Fig 9: *In vitro* effect of bioagents on growth and inhibition *Alternaria brassicae* (*Trichoderma harzianum)*

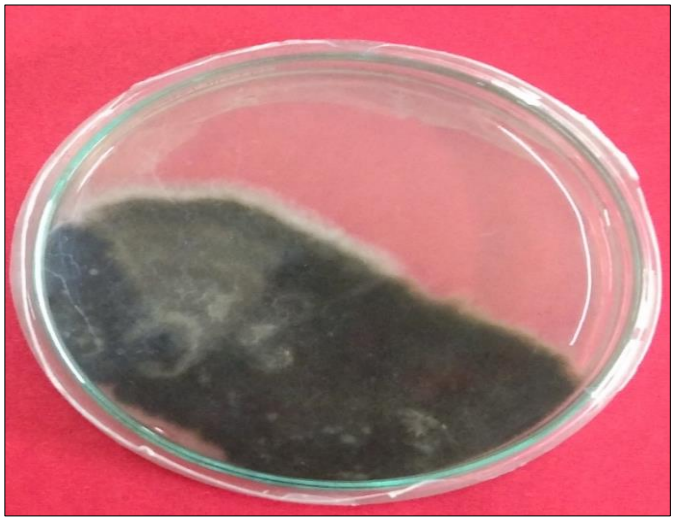

Fig 10: Control

Keeping in view, the economic importance of Mustard and losses incurred by the *Alternaria blight* (*Alternaria brassicae*) diseases, present investigation on the aspects *viz*, and *In vitro* evaluation of selected botanicals and bioagents against the pathogens were undertaken during the Rabi, 2020 at the Department of Plant Pathology, College of Agriculture, Lovely Professional University, Phagwara (Punjab). The results obtained on the above aspects during the present Investigation are being discussed herein the following paragraph.

(a) Radial Mycelial growth

Results from the (Table1) indicate that all the botanicals/ plant extracts tested exhibited a varied range of radial mycelial growth of the test pathogen. Depending upon their concentration it was ranged in increased or decreased in botanicals tested.

- Mean percentage mycelial growth inhibition recorded with all the botanicals tested was ranged from 41.79%. However, *Ginger* was found most fungi static and it is recorded with highest mean mycelial growth inhibition (41.79%). And the second-best plant extract was found to be in the range of 41.54% Tulsi, and the lowest mycelial growth was recorded with Neem that is 41.26%.
- Thus, all the plant extract tested were found to be the fungi static and antifungal against *Alternaria brassicae* and significantly inhibited its mycelial growth over untreated control. Finally, Ginger was found to be the highest Mycelial growth that (41.79%) followed by Tulsi and Neem.

(b) *In vitro* evaluation of Bio-agents

- Results from the (Table2) revealed that all the bioagents exhibited fungi static activity and significantly inhibited mycelial growth of *Alternaria brassicae* over untreated control. Of the two bioagents tested, *Trichoderma viridae* was found to be the most effective which recorded significantly least linear mycelial growth that is (19.16mm) and corresponding highest mycelial growth inhibition (20.69%) of the test pathogen over untreated control that is (24.16). and the second and the third best bioagents that is *Trichoderma harzianum* which is recorded highest Mycelial growth that is (20.5 mm) and corresponding Mycelial growth inhibition that is (15.14%) of the test pathogen of the untreated control over (24.16 mm).
- Thus, all the fungal bioagents evaluated *in vitro* were found to be fungi static against *Alternaria brassicae*

Conclusion

Based on the observation, we finally come to know that the Ginger was found to be the highest Mycelial growth that (41.79%) followed by Tulsi and Neem. Results from the (Table 2) revealed that all the bioagents exhibited fungi static activity and significantly inhibited mycelial growth of *Alternaria brassicae* over untreated control. Of the two bioagents tested, *Trichoderma viridae* was found to be the most effective which recorded significantly least linear mycelial growth that is (19.16 mm) and corresponding highest mycelial growth inhibition (20.69%) of the test pathogen over untreated control that is (24.16). and the second and the third best bioagents that is *Trichoderma harzianum* which is recorded highest Mycelial growth that is (20.5 mm) and corresponding Mycelial growth inhibition that is (15.14%) of the test pathogen of the untreated control over (24.16 mm). Thus, all the fungal bioagents evaluated *in vitro* were found to be fungi static against *Alternaria brassicae.*

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