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Influenced of natural products and bio-fungicide against tikka disease of groundnut caused by *Cercospora* spp.

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Abstract
Leaf spots are the most serious diseases of groundnut (*Arachis hypogaea* L.) on a worldwide scale. The two fungi commonly involved are *Cercospora arachidicola* Hor causing early leaf spot, and *Cercospora personatum* (Berk. & Curt.). A field trail was conducted to efficacy the effectiveness of botanicals namely neem oil, garlic oil and onion oil and bio-control agents *Trichoderma harzianum* and *Pseudomonas fluorescens* against leaf spot disease (tikka) of groundnut caused by *Cercospora* Spp. *Pseudomonas fluorescens* @ 5% foliar spray led to a significant reduction of disease incidence (20.1%) followed by *Trichoderma viride* @ 5% (FS) (22.19%), neem oil @ 5% (FS) (23.29), garlic oil @ 4% (FS) (23.70), onion oil @ 4% (FS) (24.4) compared to treated and untreated control (27.90 and 20.12). Maximum cost benefit ratio was recorded *Pseudomonas fluorescens* @ 5% (FS) 1:2.05.

Keywords: *Cercospora* spp. Groundnut, Neem oil, *Pseudomonas fluorescens*, *Trichoderma harzianum*

Introduction
Groundnut (*Arachis hypogaea* L.) is one of the most important oil seed crops of the world. Groundnut (2n=40) is unique among all the leguminous crops, designated as "wonder legume" and belongs to leguminous family**.** It is a multipurpose and highly nutritious crop containing oil, food and its foliage or haulm provides a valuable fodder for livestock. It contains about 48.32% oil, 22-25% protein, 20% carbohydrate, 5% fibre and ash, vitamin B and E. Groundnut is the 13 most important food crop of the world. China, India, United States, Nigeria, Burma, Argentina and Indonesia are the major producers of groundnut globally. Groundnut production in *Kharif* (2015-16) was 26.20 lakh tonnes. The major producers of groundnut are Gujarat (26.34%), Andhra Pradesh (19.08%), Rajasthan (17.68%), Tamil Nadu (9.54%), Karnataka (7.63%), Madhya Pradesh (7.25%) and Maharashtra (5.34). Among the fungal diseases of groundnut, tikka diseases are most common. Early leaf-spot caused by *Cercospora arachidicola* (Agrihunt, 2011) [1] and late leaf-spot due to *Cercospora personatum* (Meena, 2011) [8] are mainly prevalent during the *Kharif* season almost all groundnut growing areas of the world and become endemic frequently (Ghewande and Misra, 1983) [4]. Yield losses caused by leaf-spots ranged from 15 to 80 per cent. Losses in pod yield can be up to 29 per cent (Siddaramaiah *et al.,* 1977) [11]. Many research workers have tried to find out safe and economical control of plant diseases by using extracts of different plant parts (Bdliya and Alkali, 2008) [3]. The use of plant extracts with antifungal activity offer economical, safe and easily available alternative methods for the management of leaf spot in groundnut. Different botanicals have been found to inhibit the groundnut disease of *C. arachidicola* and *C. Personatum* (Alam *et al*., 2002) [1]. In view of the above information, it is clear that research work on leaf spot disease caused by *C. arachidicola* and *C.personatum* which cause serious problem of groundnut cultivation in the country is essential for control by plant extracts and biological control means instead of using chemicals to avoid environmental pollution.

Material and methods
Experimental site: The experiment was conducted in Farmer Experiment Field at Saharanpur Uttar Pradesh during *kharif* season in the year 2015-2016. The selected field area was well prepared and plot marked as per the lay out plan of the experiment. The selected field was dug up, cleaned and the soil was pulverized after which the total area was divided in to sub-plots.

Identification of pathogen
The diseased leaf samples of groundnut having characteristic symptoms developed on leaves were collected from the experiment field. The diseased leaf samples were cut from the plant and leaf was scraped very small portion from the infected part and temporary slides were prepared and observed under a compound microscope. Based on morphological characteristics of the fungus, especially morphology of conidia and conidiophores the pathogenic fungus causing leaf spot was identified *Cercospora arachidicola* and *Cercosporidium personatum.*

Pseudomonas fluorescens
Pseudomonas fluorescens is aerobic, gram-negative bacteria, ubiquitous in agricultural soils, and are well adapted to growing in the rhizosphere. *Pseudomonads* possess many traits that make them well suited as bio control and growth-promoting agents

Mechanism of action
The traits related to bio control include the ability to rapidly utilize seed and root exudates, colonize and multiply in the rhizosphere and spermosphere environments and in the interior of the plant, produce a wide spectrum of bioactive metabolites (antibiotics, siderophores, volatiles, and growth-promoting substances), compete aggressively with other microorganisms; and adapt to environmental stresses.

Trichoderma viride
Characteristics *Trichoderma* is a genus of asexually reproducing fungi that are often the most frequently isolated soil fungi; nearly all temperate and tropical soils contain 101–103 cultivable propagules per gram. These fungi also colonize woody and herbaceous plant materials, in which the teleomorph (genus *Hypocrea*) has most often been found. However, many strains, including most bio control strains, have no known sexual stage. They show a high level of genetic diversity, and can be used to produce a wide range of products of commercial and ecological interest. Several strains belonging to the genus *Trichoderma* have been identified so far as bio control agents of plant diseases and nematodes and a few of them have been developed and registered as bio fungicides.

Mode of action
Trichoderma strains may have one or all mechanisms of action according to species and strain. In some strains prevails the direct, hyper-parasitic activity against pathogens, in others the induction of resistance mechanism prevails or they compete with the pathogens for space and nutrients. Trichoderma strains produce a great variety of lytic enzymes most of which play a great role in bio control (cell wall degrading enzymes, CWDEs). CWDEs from different *Trichoderma* strains showed antifungal activity towards a broad spectrum of fungal pathogens Trichoderma produces also many secondary metabolites, some of them specifically involved in the direct antibiosis, like i) volatile antibiotics, i.e. 6-pentyl-a-pyrone (6PP) and most of the isocyanides derivates water-soluble compounds, *i.e.* heptelidic acid or koningic acid peptaibols, which are linear oligo peptides of 12–22 amino acids rich in a-amino isobutyric acid, N-acetylated at the N-terminus and containing an amino alcohol (Pheol or Trpol) at the Cterminus. The production of lytic enzymes and antibiotic metabolites may greatly vary among strains. Each strain shows specific characteristics and adaptation to the environment. Some strains are very good colonizers of soil other prefer wood material, others are found on dead plant tissue in the phyllosphere or colonizing the rhizosphere of plants and the root tissues. Several strains can also promote growth.

Application of spray solution: Botanicals and bio control agents were sprayed as solution into the experimental plots as per treatments. Spraying was done for 3 times with 10 days interval at 65, 75 and 85 DAS respectively. Adequate precautions were taken to avoid drifting of spray materials from one plot to the neighboring ones. Plants were graded in 1-9 scale. Per cent disease intensity (PDI) was worked out using the following formula:

$$\text{PDI \%} = \frac{\textbf{Sum of all numerical ratings}}{\textbf{Total plants observed x Maximum ratings}} \times 100$$

The PDI values were transformed by square root transformation and angular transformation and analysed statistically. The per cent disease control was calculated using formula:

$$\text{PDI\%} \frac{\textbf{Disease intensity (\%) in control} - \textbf{Disease intensity (\%) in treatement}}{\textbf{Disease intensity (\%) in control}} \times 100$$

Benefit – cost analysis (BCR): The Benefit-Cost analysis was done following the method of Mondal *et al.* (1994) [10].

$$\text{BCR} = \frac{(A \times C) - B}{B}$$

Where,
A = Selling price (Tk./kg)
B= Cost of cultivation of the crops (Tk./ha)
C= Yield (Kg/ha)

Pathogenicity test
Pathogenicity test of *Cercospora* spp. was carried out on healthy detached groundnut leaves. Fresh groundnut leaves infected with tikka disease were collected from the green house as well as from the field. Superficial fungal growth was scraped with blade and incorporated water. The spore suspension (4 ×105 spore/ml) thus, prepared was utilized for the experiment

Experimental design and statistical analysis
The experiment was done following Randomized Complete Block Design (RCBD) with three replications. The experimental field was primarily divided into 7 blocks. Each block was further divided into 3 plots. Total number of plots was 21.

Result and discussion
Effects of biotic and botanicals inducers on groundnut crop
Plant height and number of pods per plant affected by different treatments at 60 DAS
All treatments significantly increased the plant height (cm) of groundnut plant as compared to the control either as treatment. Maximum plant height was recorded in *Pseudomonas fluorescens* (37.36) as compared to treated and untreated control (28.36 and 25.99, respectively). The second

best treatment was *Trichoderma viride* (34.16), which was followed by neem oil (31.27), garlic oil (30.46) and onion oil (29.59) as compared to untreated control (26.00). Among the treatments most effective was *Pseudomonas fluorescens* (37.36). The highest number of pods was recorded *Pseudomonas fluorescens* (38.11) as compared to treated and untreated control (30.01 and 21.73, respectively). The second best treatment was *Trichoderma viride* (36.12), which was followed by neem oil (34.02), onion oil (33.40) and garlic oil (33.42) as compared to untreated control (21.73). Among the treatments most effective was *Pseudomonas fluorescens* (38.11**).** Similar observations have also been made by Hossain *et al.,* 2009 [5].

Number of leave per plant affected by different treatments 60 DAS

Among the bio-agents and botanicals used the number of leave per plant was recorded in *Pseudomonas fluorescens* (187.35) as compared to treated and untreated control (165.18 and 160.54, respectively). The second best treatment was *Trichoderma viride* (184.36), which was followed by onion oil (183.57), neem oil (183.36) and garlic oil (181.45) as compared to untreated control (160.54). Among the treatments most effective was *Pseudomonas fluorescens* (183.35). Use of bio fungicide also increase plant growth was reported by Mishra and Sinha, 2002[9].

Table 1: effect of bio-agents and botanicals on plant height, number of leaves, weight of pods, number of pods per plant, disease incidence percent and yield (q/ha)

Treatments	Plant height 60 DAS (cm)	No. of leave per plant 60 DAS	Weight of pods per plant (gm)	No. of pods per plant	Yield (q/ha)	Disease intensity percent90 DAS	C:B ratio
Trichoderma viride	34.16	184.36	25.90	36.12	27.07	22.19	1:1.90
Pseudomonas fluorescens	37.36	187.35	28.12	38.11	28.11	20.11	1:2.05
Neem oil	31.27	183.46	19.91	34.02	25.95	23.29	1:1.50
Garlic oil	30.46	181.45	18.88	33.42	24.02	23.70	1:1.45
Onion oil	29.59	183.57	18.98	33.40	25.11	24.04	1:1.35
Carbendazim (Treated control)	28.66	165.18	18.13	30.01	27.90	21.09	1:1.80
Control (Untreated)	25.99	160.54	16.48	21.73	20.12	42.02	1:1.10
C. D.(P= 0.05%)	1.002	3.127	0.842	0.981	0.924	0.966	-

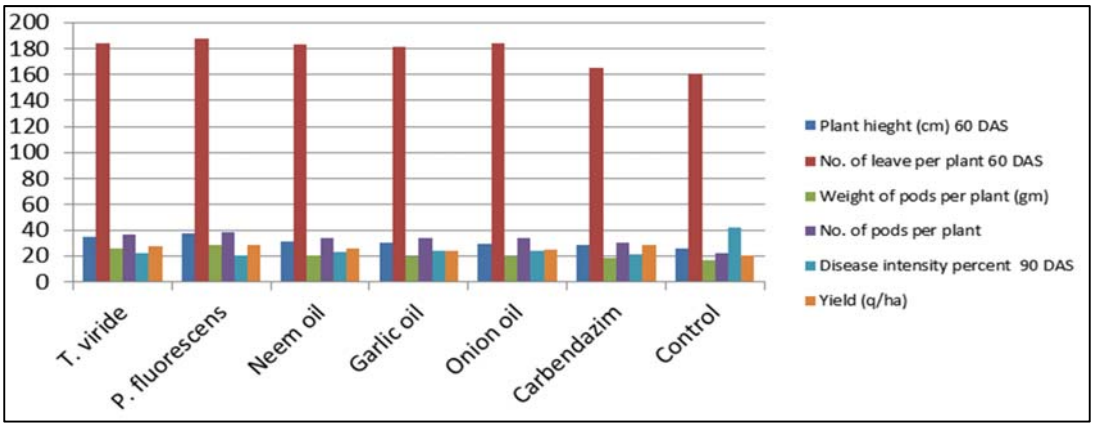

Fig 1: Effect of bio control agents and botanicals on plant height, number of leaves per plant, weight of pods, number of pods per plant, disease intensity and yield (q/ha)

Weight of pods per plant (g) affected by different treatments

Among the bio-agents and botanicals used the weight of pods per plant was recorded in *Pseudomonas fluorescens* (28.12) as compared to treated and untreated control (18.13 and 16.48, respectively). The second best treatment was *Trichoderma viride* (25.90), which was followed by neem oil (19.91), garlic oil (18.88) and onion oil (18.98) as compared to untreated control (16.48). Among the treatments most effective was *Pseudomonas fluorescens* (28.12).

Disease intensity per cent at 90 DAS

The incidence of the diseases was found to be higher in the control than in the treated by*Pseudomonas fluorescens* (20.11) as compared to treated and untreated control (21.09 and 42.09, respectively). The second best treatment was *Trichoderma viride* (22.19), which was followed by neem oil (23.29), garlic oil (23.70) and onion oil (24.04) as compared to untreated control (42.09). Among the treatments most effective was *Pseudomonas fluorescens* (20.11). These results are similar with the observations of Mathivanam *et al.* (2000) and Meena *et al.* (2000) [7]

Yield of groundnut (q/ha) as influenced by different treatments.

The data presented in (Table 1) revealed that maximum yield of groundnut (q/ha) was recorded in *Pseudomonas fluorescens* (28.11) as compared to treated and untreated control (27.90 and 20.12, respectively). The second best treatment was *Trichoderma viride* (27.07), which was followed by neem oil (25.95), garlic oil (24.02) and onion oil (25.11) as compared to untreated control (2.12).

Cost benefit ratio

Data was presented (Table 1) maximum cost benefit ratio was recorded in *Pseudomonas fluorescens* (1:2.05) as compared to

treated and untreated control (1:1.80 and 1:1.10, respectively). The second best treatment was *Trichoderma viride* (1:1.90), which was followed by neem oil (1:1.50), garlic oil (1:1.45) and onion oil (1:1.35) as compared to untreated control (1:1.10). The present investigation indicates that application of *P. fluorescens, T. harzianum* can be used as an effective treatment of tikka disease and to develop ecofriendly strategy for the management of tikka disease of groundnut

Conclusion

In the present study, on the basis of observation, it was found that for controlling leaf spot diseases of groundnut. *P. fluorescens* @ 5 per cent (20.11) was the best effective in comparison to other treatments followed by *T. viride* (22.19) as effective next to chemical and even the cost benefit ratio of *P. fluorescens* (1:2.05) treatment. Hence, from the present study it can be concluded that *P. fluorescens* comparison to chemical fungicides Carbendazim, can be used effectively to reduce the disease intensity as both are non-significant to each other and got better yield similar to that with the use of chemicals.

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