

## **Effect of Number of ULV Application of Neem Seed Oil on the Incidence of Cercospora Leaf Spot and Seed Yield of Sunflower (*Helianthus annuum* (L.))**

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**Abstract:** *The field experiment was conducted during the rainy season of 2017 cropping season at the Mohammed Lawan College of Agriculture Teaching and Research farm to evaluate the effect of number of ULV application of Neem seed oil on the incidence of Cercospora leaf spot and yield of Sunflower (*Helianthus annuus* L.) was tested in field trial. The trial was laid using randomized complete block design with five replicates. Result showed that, there was significantly higher incidence of the disease on unsprayed (check) plot than on other treatments. Disease incidence among plants sprayed once in three weeks and two weeks with Neem seed oil and with Sevin 85WP also sprayed once in two weeks were not significantly different. Plants sprayed weekly had the lowest disease incidence. There was significantly higher seed yield by those plants sprayed weekly. Those sprayed either once in two weeks with Neem seed oil and Sevin 85 WP or once in three weeks were not significant. The unsprayed plot had the lowest seed yield.*

**Key words:** *ULV, Neem seed oil, Cercospora leaf spot, Seed yield, Sunflower*

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### **INTRODUCTION**

Sunflower (*Helianthus annuus* L.) is one of four main annual oil plants that is cultivated for its oil and nut varieties (Mehrabi and Pakravan, 2009). The plant has an important and industrial food product, because of its nutritional features and the potential for earning exchange has become a valuable product in foreign and inner markets and has a special position in agricultural sector (Demirci and Kaya, 2009; Bagherzadeh, 2010). In 19<sup>th</sup> century, the cultivation of sunflower as an oil seed crop began in the Soviet Union and the majority of the present day varieties grown all over the world trace back their origin to the U.S.S.R (Semerci, 2013). In 1985 – 86 Sunflower seed was the third largest source of vegetable oil worldwide following Soybean and palm (Putnam *et al.*, 1990; Semerci, 2012). In Africa, Tanzania is the largest producer with 10,000 – 20,000 tons per annum; other small producers are Kenya, and Zimbabwe (Purseglove, 1991). After series of preliminary investigation and field studies on Arable oil seed started in 1965, Sunflower was identified as a potential alternative source of vegetable oil in Nigeria (Mshelia and Sajo, 2012). Anonymous (2013), confirmed that sunflower fit well into Nigerian farming besides being a

suitable alternative of vegetable oil.

Sunflower can be grown on a wide range of soil but should be sandy loam, black soil, and alluvial soil with optimum pH of 6.0 – 8.5 (Kaya *et al.*, 2009). However, the crop is been affected by many disease pathogen notably the *Cercospora* leaf spots. *Cercospora leaf spots* are fungus disease which responsible for the diseases on the various hosts (field crops). The fungus produces long, slender, color less, to dark, straight to slightly curved, multicellular conidia on short dark conidiophores (Gungor and Semerci, 1999; Semerci, 2011). Conidiophores arise from the plant surface in clusters through the stomata and form conidia successively on new growing tips. Conidia are detached easily and are often blown long distances by the wind (Bayramoglu *et al.*, 2005). The margin of the spots remains purple or brown giving the lesion a “bird’s eye “appearance. Infection sites appear as indistinct tan or bluish areas on lower leaf surface. Symptoms expression is variable depending on the age of the leaf, susceptibility of the cultivar or species, fungal strain and environmental conditions. Leaf spot may reach economic threshold levels, provided young leaves and inoculum are present, under conditions of high temperature and long period of leaf wetness (Devarajan *et al.*, 1988; Brahmachari, 2004).

Research result show most severe infection of young leaves to occur during periods of leaf wetness from 12 to 96 hours when temperatures fall in the range of 15-20°C (Adivar, 2004). The fungus is favored by high temperatures and therefore is most destructive in the summer months and in warmer climates (Rahman and Hossain, 2005). Most *Cercospora* species produce the nonspecific toxin cercosporin, which acts as a photosensitizing agent in the plant cells; it kills cells only in the light (Abdulraman and Alkhaili, 2005). The toxin incites the production of reactive atomic oxygen in the cells (Kishore and Pande, 2005). Although *Cercospora* spores need water to germinate and penetrate, heavy dews seem to be sufficient for infection (Yun *et al.*, 2013; Gosse *et al.*, 2005). The pathogen over seasons in or on the seed and as minute black stomata in old infected leaves (Aage, 2003).

However, modern protection of field crops against pathogens is seeking to apply some of nontoxic materials such as botanical aqueous extract, oils and powders which are increasingly becoming an integral part of programs for control of diseases (Gopal *et al.*, 2006). Neem seed oil are very promising alternatives to traditional residual fungicides (Hossain *et al.*, 2005). They are of natural origin, have negligible toxicity to mammals and can be applied with similar technology to that needed for residual pesticides (Ihejirika *et al.*, 2006). Neem seed oil has a unique, broad-spectrum mode of action against many insect pests and diseases. Products based on neems are highly effective even at low application rates (Suleiman and Omafè, 2013; Zang *et al.*, 2010). However, *Cercospora* disease can be controlled by using disease-free seed or seed least than three years old, by which time the fungus in the seed has died; by using crop rotations with hosts not affected by the same *Cercospora* species; and by spraying the plants, both in the seed bed and in the field, with appropriate fungicides (Natarajan, 2005). The objective of the study is to evaluate effect of neem seed oil in managing *Cercospora* leaf spot of sunflower.

## **MATERIALS AND METHOD**

Field trial was conducted at the Mohamet Lawan College of Agriculture Teaching and Research farm, latitude 11°50N and longitude 13°05N in 2014 cropping season. The experiment consist of 25 sub-plots measuring 5x10 meter, the design used was split plot arrangement and the treatment were replicated five times, the whole experimental area

was measuring 29 x 50 m<sup>2</sup>. The seed were planted in the month of July, 2015 with inter - row spacing of 70 cm and intra- row spacing of 45 cm, the seed were treated with Apron star 42WS at the rate of 2g/kg seed to control soil borne pathogen and pest. A compound fertilizer N.P.K 15:15:15 was applied at four weeks old and the second dose were applied at six weeks old after planting.

### PARAMETERS AND DATA COLLECTION

Data were collected on seedling emergence, incidence of Cercospora disease and yield of sunflower after harvesting respectively. The experiment was made up of five treatments viz: one spray in one week, one spray in two weeks, one spray in three weeks, and one spray in two weeks (using sevin 85WP) and check (unsprayed).

### RESULT AND DISCUSSIONS

**Table 1: Spray schedule of neem seed oil and Sevin 85Wp for protection of sunflower against Cercospora leaf spot.**

<u>Treatment</u>	<u>Days after flowering</u>					<u>Total number of sprays applied</u>
	42	49	56	63	70	
<b><u>Neem seed oil</u></b>						
One spray in one week	+a	+	+	+	+	5
One spray in two weeks	+	-	+	-	+	3
One spray in three weeks	+	-	-	+	-	2
<b><u>Sevin 85Wp</u></b>						
One spray in two weeks	+	-	+	-	+	3
<b><u>Check (Unsprayed)</u></b>	-b	-	-	-	-	0

a = sprayed applied.

b = no spray applied (unsprayed).

The effect of number of ULV application of neem seed oil on the incidence of Cercospora leaf spot and seed yield of sunflower, the study investigated the effect of interval of spray of neem seed oil on the incidence of the disease. The results in table 1 showed that there was lower disease incidence if the interval of spray was small. This implied that with the lowest incidence and the highest yield obtained when spray interval was small, any delay in spraying resulted in higher disease incidence and lower seed yield. A similar finding was obtained on the effect of severity of damage by *Podagrica species*, and *Helicoverpa armigera* on Okra (Anaso and Lale, 2002).

**Table 2: Mean incidence of Cercospora leaf spot on sunflower as affected by interval of ULV spray of neem seed oil.**

Interval spray	Percentage of Cercospora leaf spots
Once every week	18.5 <sup>c</sup>
Once every two weeks	20.2 <sup>bc</sup>
Once every three weeks	22.7 <sup>ab</sup>
Sevin 85Wp at once every weeks	21.4 <sup>bc</sup>
Unsprayed (check)	20.4 <sup>a</sup>
Mean	21.8
CV (%)	9.41
S.E ±	0.92**

\*\* Significant at ( $p \leq 0.001$ ) mean with the same letter (s) are not significantly different according to Duncan's multiple range test (DMRT).

Table 2, also showed that the disease incidence among plants sprayed once three and two weeks and those sprayed with sevin 85wp once in two weeks were not significantly different, although the plants sprayed weekly had the lowest incidence of Cercospora leaf spot, the differences between them and those sprayed once in two weeks and those sprayed with sevin 85wp were not significant.

**Table 3: Mean seed yield of sunflower as affected by interval of spray of Neem seed oil.**

Interval of spray	Seed yield (kg/ha)
Once every week	1,096 <sup>a</sup>
Once every two weeks	1,008 <sup>ab</sup>
Once every three weeks	788 <sup>bc</sup>
Sevin 85Wp sprayed once every two weeks	1020 <sup>ab</sup>
Unsprayed (check)	580 <sup>c</sup>
Mean	900
CV (%)	16.1
S.E ±	401 **

\*\* , Significant at ( $p \leq 0.001$ ) mean with the same letter (s) are not significantly different according to Duncan's multiple range test (DMRT).

Table 3, showed the effect of interval of spray of neem seed oil on the seed yield of sunflower. There was significantly higher seed yield by plants sprayed with neem seed oil every two weeks than other treatments except sprayed once every two weeks and those sprayed with sevin 85wp also every two weeks. The unsprayed plants had the lowest seed yield except that the difference between them and those sprayed with neem seed oil every three weeks was not significant. The findings also reveals that the difference in seed yield among plants sprayed twice and three times every week with neem seed oil and those sprayed two times a week with sevin 85wp were not significant.

## DISCUSSION

The results of this study showed that neem seed oil had antimicrobial properties against *Cercospora* leaf spot. Many workers have reported the use of plant extracts in the control of fungal diseases (e.g. Dubey *et al.*, 2009; Satish *et al.*, 2008). Many phytofungicides have been obtained from a number of plant extracts. These include "Fitoekols-IF" from *Pinus sylvestris* and *Picea abies* greens extract, "Fitosativum" from *Allium sativum* extract, "Fitocapsicum" from *Capsicum annuum* extract, "Fitokrisanthemium" from *Chrysanthemum* sp. leaf extract, "Fitoarmoracium" from *Armoracia rusticana* root and leaf extract, "Fitotabacum" from *Nicotiana tabacum* and *N. rustica* extracts, "Fitopelargonium" from *Pelargonium* sp. leaf extract and "Fitosinepium"-from white mustard (*Sinapis alba*) plant and seed extract (Zarins *et al.*, 2009). Citrus fruits have also been acknowledged by Munoz and Marcos (2006) to possess a variety of phytofungicides that help to inhibit fungal growth and development. Afzal *et al.* (2010) reported *Allium sativum* to have a wide antifungal spectrum that affects 60-82% inhibition in the growth of seed borne *Aspergillus* and *Penicillium* fungi. This was attributed to phytochemical properties of garlic plant, allicin which could decompose into several effective antimicrobial compounds such as diallyl sulphide, diallyl disulphide, diallyl trisulphide, allyl methyl trisulphide, dithiols and ajoene (Salim 2011; Tagoe, 2011).

According to Mulla and Su (1999) and Biswas *et al.* (2002), neem oil, extracted from the seeds of *Azadirachta indica*, has versatile medicinal properties, including antifertility, antifungal, antibacterial, immunostimulant, antipyretic and acaricidal activities. Chloroform extracts and petroleum ether extracts of neem oil have also been found to exhibit potent acaricidal activity against *Sarcoptes scabiei* var. *cuniculi* larvae (Du *et al.*, 2008, 2009). Neem extract was also found by Da-Costa *et al.* (2010) to have inhibited the fungal growth (*i.e.* mycelia dry weight, diameter of colony and growth rate) of *Aspergillus flavus* on solid media at concentrations from 0.5 to 5.0% v/v, although it significantly increased sporulation in the same conditions. Bhutta *et al.* (2001) tested 32 different seed diffusates against *Aspergillus alternata* and *Fusarium solani* and found that the diffusates from *Coriander sativum* and *Memoranda charata* exhibited inhibitory effects at 0.5% and 1% concentrations. Eksteen *et al.* (2001) also tested 11 plant extracts against different pathogenic fungi including *F. oxysporum* and *Rhizopus solani* by the agar dilution method and obtained encouraging results comparable inhibitory effects on mycelial growth with reference to those obtained using Carbendazim and Difenconazole. Similar observations were recorded against *Alternaria solani* by using *Allium cepa* extract (Khallal, 2001).

Locke (1995), Martinez (2002) and Da-Costa *et al.* (2010) all reported that due to the antifungal efficacy of neem seed extract, its biodegradability and minimum side effects, azadirachtin, a tetranortriterpenoid obtained from the seed has emerged as a natural

biopesticide. In addition, the percentage inhibition against the tested fungi were found to increase at different rates by increasing the concentration of neem leaf and seed extract with the result that neem seed organic extracts had higher inhibition percentage than that of neem leaf organic extracts. The result further indicates that prompt application and decreased in number of days of applications is more effective for control of *Cercospora* leaf disease for obtaining high yield. The findings was in agreement with the findings of Aage *et al.* (2003). Similar results have also been reported by Natarajan *et al.* (2005), Kishore and Pande (2005) and Abdulrahman and Alkhali (2005) reported that weekly or frequent application of NSO give better result and high yield in return than delayed in spraying intervals. The present results also support the findings of Adiver (2004), Gopal *et al.*, (2006) and Ihejirika *et al.* (2006).

### CONCLUSION

Results of the present investigation provide evidence that neem seed oil has an antifungal activities and is effective against the control of *Cercospora* leaf spot of sunflower and maximum yield could be obtained when the crop are sprayed on weekly interval and then followed by other agronomic practices.

### REFERENCES

- Aage, V. E., S. J. Gaikwad, G. T. Behere & V. S. Tajame. (2003). Efficacy of extracts of certain indigenous medicinal plant against *Cercospora* leaf spot of groundnut. *Journal of Biological science* (6): 576-581.
- Abdulrahman, A. and Alkhaili, Aba. (2005). Antifungal activity of some extracts against some plant pathogenic fungi. *Pakistan J. Biol. Sci.* **8** (3): 413-417.
- Adivar, S. S. (2004). Field efficacy of botanical preparations on late leaf spot (*C. Personata*) of groundnut (*Arachis hypogaea*). *Oil Seed Res.* **21** (2): 369-370.
- Afzal, R., S.M. Mughal, M. Munir, S. Kishwar, R. Qureshi, M. Arshad & Laghari, M.K. (2010) Mycoflora associated with seeds of different sunflower cultivars and its management. *Pakistan Journal of Botany.* 42: 435-445.
- Anaso, C. E & Lale, N.E.S (2002): Spray interval and cost benefit of using aqueous neem kernel extract and deltamethrin against some foliage fruit pest of Okra in Sudan savannah of Nigeria. *Journal of sustainable Agriculture and the Environment* 4(1): 122-128.
- Anonymous, (2013). Food and Agricultural Organization of the United Nations, Statistical Database.
- Bagherzadeh, A. (2010), Comparative Analysis of Economic-Social Features on Technical Efficiency of Sunflower Farms (The case of Khoy sunflower), Agricultural Sciences of Islamic Azad University of Khoy.
- Bayramoglu, Z., Goktolga, Z. G. & Gunduz, O. (2005). Physical Production Inputs and Cost Analysis of Some Important Field Crops in Zile County of Tokat Province, *Turkish Journal of Agricultural Economics* 11 (2) 101 – 109.
- Bhutta, A.R., Bhatti, M.H.R., Ahmad, I. and Sultana, I. (2001). Chemical control of seed borne pathogens of sun flower. *Helia*, 24(35):67-72.
- Biswas, K., Chattopadhyay, I., Banerjee, R.K., Bandyopadhyay, U. (2002). Biological activities and medicinal properties of neem (*Azadirachta indica*). *Current Sciences* 82 (11),

1336–1345.

- Brahmachari, G. (2004). Neem – an omnipotent plant: A retrospection. *Journal of Chemistry and Biological Chemotheraphy*. 5: 408–421.
- Culbreath, A. K., Stevenson, K. L & Brenneman. T. B. (2002). Management of late leaf spot of peanut with benonyl and chlorothalonil: A study in preserving fungicide utility. *Plant Dis*. 56: 349-355.
- Da-Costa Christiane L., Marcia R. F. Geraldo, Carla C. Arrotéia, Carlos Kimmelmeier. (2010). *In vitro* activity of neem oil [*Azadirachta indica* A. Juss (*Meliaceae*)] on *Aspergillus flavus* growth, sporulation, viability of spores, morphology and Aflatoxins B1 and B2 production. *Advances in Bioscience and Biotechnology*, 1: 292-299.
- Demirci, M. & Y. Kaya, (2009). Status of *Orobanche cernua* (Loefl) and weeds in sunflower production in Turkey. *Helia*, 32 (51): 153-160.
- Devarajan, R; Kumarasan, K.R.J., Ramanathan, G.J. & Pachananthan, R. M (1988): Response of Sunflower to micronutrients. *Madras Agricultural Journal* 75(11-12)401 – 409(En, 4 ref.) Dept. of Soil Science. Tamil Nadu 64 1003 India
- Du, Y.H., Jia, R.Y., Yin, Z.Q., Pu, Z.H., Chen, J., Yang, F. & Zhang, Y.Q. (2008). Acaricidal activity of extracts of neem (*Azadirachta indica*) oil against the larvae of the rabbit mite *Sarcoptes scabiei* var. *cuniculi* in vitro. *Veterinary Parasitology*, 157, 144–148.
- Du, Y.H., Li, J.L., Yin, Z.Q., Li, X.T., Jia, R.Y., Lv, C., Zhang, Y.Q., Zhang, L. (2009). Acaricidal activity of four fractions and octadecanoic acid-tetrahydrofuran-3, 4-diyl ester isolated from chloroform extracts of neem (*Azadirachta indica*) oil against *Sarcoptes scabiei* var. *cuniculi* larvae in vitro. *Veterinary Parasitology*, 163 (1–2), 175.
- Dubey, R.C., Kumar, H. & Pandey, R.R. (2009). Fungitoxic effect of neem extracts on growth and sclerotial survival of *Macrophomina phaseolina* in vitro. *Journal of American Science*, 5(5):17-24.
- Eksteen, D., Pretorius, J.C., Nieuwoudt, T.D. & Zietsman, P.C. (2001). Mycelial growth inhibition of plant pathogenic fungi by extracts of South African plant species. *Annals of Applied Biology*, 139 (2):24-39.
- Gopal, K., S. K. Ahamed & G. P. Babu. (2006). Estimation of losses due to tikka leaf spot in groundnut (*Arachis hypogaea* L.). *Legume-Res*. 29 (4): 289-291.
- Gossé, B., Amissa, A.A. & Adjé, F.A. (2005). Analysis of components of neem (*Azadirachta indica*) oil by diverse chromatographic techniques. *J. Liquid Chromatogr. Relat. Technol.*, 28: 2225–2233.
- Hossain, M. S., Khalequzzaman, K. M. Hossain, M. A Hossain, M & Mollah., M. R. A (2005). Screening of groundnut genotypes for leaf spots and rust resistance. *J. Subtropical Agril. Res. and Develop*. 3(1): 57-60.
- Ihejirika, G. O., Nwufu, M. I., Duruigbo, C. I., Onwerenadii, O. P., Obili, E. R. Omuoha K. O & Ogbede, S. (2006). Effect of plant extracts and plant density on the severity of leaf spot disease of groundnut. *J. Plant Sci*. 1 (4):374-377.
- Kaya, Y., G. Evcı, V. Pekcan, T. Gucer, & Yilmaz, I.M. (2009). The evaluation of broomrape resistance in sunflower hybrids. *Helia*, 32 (51): 161-170.
- Kishore, G. K & Pande, S. (2005). Integrated applications of aqueous leaf extract of *Datura metel* and chlorothalonil improved control of late leaf spot and rust of groundnut. *Australasian Plant Pathol*. 34 (2): 261-264.
- Locke, J.C. (1995) Fungi. In Schmutterer, H. Ed., *the Neem Tree*, VHC. Weinheim, Germany,

118-126.

- Mehrabi, H., & Pakravan, M. (2009). Calculating the Varieties of Efficiency and Returns to Scale of Sunflower Producers in Khoy, Economy & Agricultural Development. (Abstract) (In Persian). *MPM Journal*, Publication No: 1999/3, ISSN: 1013, 1388, Ankara, Turkey. Pp.193-202.
- Mshelia, J. S. & Sajo, A. A. (2012). Oil seed production for sustainable development in north eastern Nigeria, *Journal of agriculture and veterinary science*. Vol, 4.
- Muñoz, A. & Marcos, J.F. (2006) Activity and mode of action against fungal phytopathogens of bovine lactoferricin-derived peptides. *Journal of Applied Microbiology*, 101 (6) :1199-1207.
- Natarajan, D., K. Srinivasan, C. Mohanasundari, G. Perumal, M. A. N. Dheen, G. A Ganapathi & T. Rajarajan. (2005). Antifungal properties of three medicinal plant extracts against *Cercospora arachidicola*. *Advances in Plant Sciences* 18 (1): 45-47.
- Purseglove, J.W. (1991): Tropical crop Dicotyledon volume 1 and 2, 1<sup>st</sup> edition. Longman group limited pp67-73.
- Rahman, M. A. & Hossain, I. (1996). Controlling *Cercospora* leaf spot of Okra with plant extracts. *Bangladesh Hort.* 24 (1&20): 147-149.
- Salim, A.B. (2011) Effect of some plant extracts on fungal and aflatoxin production. *International Journal of Academic Research*. 3: 116-120.
- Satish, S., Raghavendra, M.P., Mohana, D.C. & Raveesha, K.A. (2008). Antifungal activity of a known medicinal plant *Mimusops elengi* L. against grain moulds. *Journal of Agricultural Technology*, 4 (1):151-165.
- Semerçi, A., (2012). Productivity analysis of sunflower production in Turkey. *Pak. J. Agri. Sci., Vol. 49 (4) 577-582*.
- Semerçi, A., (2013). Functional analysis of sunflower (*Helianthus annuus* L.) production in Turkey: A case study of Thrace region. *Journal of Food, Agriculture & Environment Vol.11 (1): 436 – 440*.
- Semerçi, A., Kaya, K. Peker, I. Sahin, Y. & Citak, N. (2011). The analysis of sunflowers yield and water productivity in Thrace region. *Bulg. J. Agric. Sci.* 17: 207-217.
- Suleiman, M.N. & Omafefe, O.M. (2013). Activity Of Three Medicinal Plants On Fungi Isolated From Stored Maize Seeds (*Zea Mays* (L.) *Global Journal of Medicinal Plant Research*, 1(1): 77-81.
- Tagoe, D., S. Baidoo, I. Dadzie, V. Kangah, H. & Nyarko, H. (2011) A comparison of the antimicrobial (Antifungal) properties of onion (*Allium cepa*), Ginger (*Zingiber officinale*) and garlic (*Allium sativum*) on *Aspergillus flavus*, *Aspergillus niger* and *Cladosporium herbarum* using organic and water base extraction methods. *Research Journal of Medical Plant*, 5: 281-287.
- Xu, J., Fan, Q.J. & Yin, Z.Q. (2010). The preparation of neem oil microemulsion (*Azadirachta indica*) and the comparison of acaricidal time between neem oil microemulsion and other formulations in vitro. *Journal of Veterinary and Parasitology*, 169: 399-403.
- Yun-xia Denga, Mei Caob, Dong-xia Shia, Zhong-qiong Yina, Ren-yong Jia a, Jiao Xua, Chuan Wang, Cheng Lva, Xiao-xia Lianga, Chang-liang Hea, Zhi-rong Yang & Jian Zhao. (2013). Toxicological evaluation of neem (*Azadirachta indica*) oil: Acute and subacute toxicity. *Environmental toxicology and pharmacology* 35: 240-246.
- Zarins, I., Daugavietis, M. & Halimona, J. (2009). Biological activity of plant extracts and their

application as ecologically harmless biopesticide. *Sodininkyst eirdar, ininkyst*, 28(3):269-280.

Zhang, Y.Q., Xu, J. & Yin, Z.Q. (2010). Isolation and identification of the antibacterial active compound from petroleum ether extract of neem oil. *Journal of Fitoterapia*, 81 (7): 747–750.