

# International Journal of Sciences: Basic and Applied Research (IJSBAR)

ISSN 2307-4531 (Print & Online)



http://gssrr.org/index.php?journal=JournalOfBasicAndApplied

# The Effect of Pre-emergent Herbicides on Soil Microflora and N-Fixing Bacteria in Pea Field

Ali M. Zaid<sup>a\*</sup>, Muftah Mayouf<sup>b</sup>, Yahya Said Farouj<sup>c</sup>

<sup>a</sup>Biotechnology Department Azzaytuna University.

<sup>b,c</sup> Plant Protection Department , Agriculture research Centre. Tripoli Libya

#### **Abstract**

To study the effects of pre-emergence herbicides on soil microflora and nitrogen fixing bacteria in peas field [Pisum sativum], Pea was grown and treated with one or a mixture of two of Five herbicides. Soil samples were collected 1 wk. after herbicides application. Average number of colony forming units per gram of soil of bacteria, actinomycetes, and soil fungi were determined. Average number of nodules per plant was obtained at the end of the growing season. The results of the study showed Terbutryn, Propyzamide, and propyzamide+Terbutryn and hand weeding had no significant effect on soil bacteria, but Carbetamide reduced the number of soil bacteria, while Metribuzin increased the population of soil bacteria. Actinomycetes were reduced by all herbicides treatments. All treatments showed no significant effects on soil fungi except Propyzamide and metribuzine increased total number of soil fungi. The herbicides used showed no significant effects on nitrogen fixing bacteria except carbetamide that increased total number of nodules per plant.

Keywords: Soil microflora; Herbicides; Legum crops

# 1. Introduction

Soil fertility usually depends on the balance of different soil microflora, including soil fungi, bacteria, actinomycetes and other soil inhabiting microorganisms. Rhizobium legume symbiosis are the major source of fixed nitrogen in land based system and can provide about 50% of the biological source of fixed nitrogen [32].

-----

\* Corresponding author.

E-mail address: amzaid2003@yahoo.com.

Atmospheric nitrogen fixed symbiotically by the association between rhizobium species and legumes represents a remarkable source of N for agriculture [30]. Legume crops are high in protein contents and has high N-needs that generally are met through a symbiotic relationship with N-fixing bacteria species, however the amount of N-fixed depends upon a number of factors including, soil fertility, crop species and variety, water availability and temperature as well as soil and crop management [18,20]. Therefore, any factor or factors that affect this relationship may have a negative impact on the N-supply to the plant .The use of herbicides have been shown to effect plant growth and have a negative effect on soil microorganisms growth and metabolism [6,10,24,31]. The use of herbicides reduced plant growth, nodulation and had a lethal effects on the symbiotic activities between rhizobium and pea plants [2,3]. Other research has shown some herbicides may directly affect rhizobium growth [19] rhizobium survival [4], rhizobium recognition of the host plant [11,12], and nodule formation [25,26] . Soil microorganisms like bacteria, fungi ,algae ,protozoa, actinomycetes, and some nematodes have a vital role in maintaining the soil productivity .Soil microbial biomass is considered an active nutrient pool to plants. The common use of herbicides may negatively affect N fixation either directly by affecting Rhizobium [4,19,21] Or indirectly by reducing Photosynthate allocation to the nodules for N fixation, or by restricting root growth and hence the number of root sites available for infection [8,16,23,29]. The objectives of the study were to evaluate the effects of pre-emergent herbicides on the soil microflora and nitrogen fixing bacteria in pea field in Libya.

# 2. Methods and materials

# 2.1 Soil samples

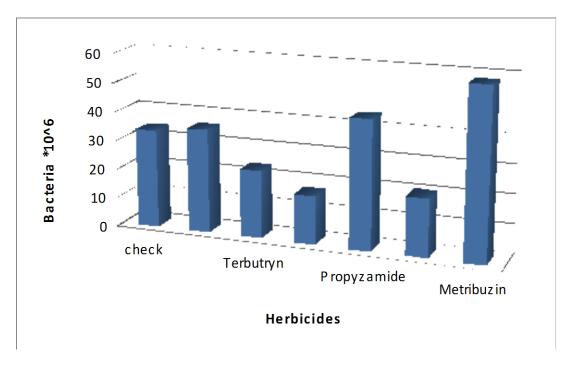
The experiment was conducted at Tajura Experimental station of the Agriculture Research Center. pea seeds were sown in plots [3x5m] with three replicates in Randomized Complete Block design. Each plot was treated with one or a mixture of two herbicides table [1]. After seven days of herbicides application, 1-2 kgs were collected from 2-3 spots at 0-20cm depth from each plot and bulked in one new plastic pack. Soil samples were transferred to the laboratory and kept in refrigerator. Soil samples were processed within 1wk.

### 2.2 Isolation procedures

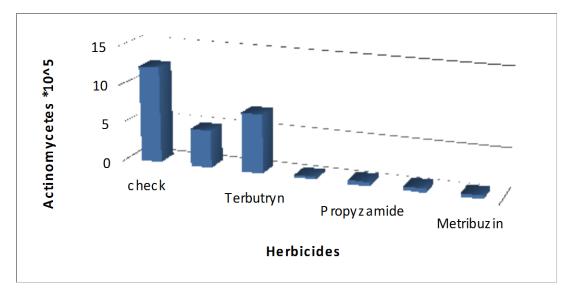
Serial dilution technique was used to isolate soil bacteria, soil fungi, and actinomycetes. Isolation was done using pour plate method. 0.1ml of a proper dilution was transferred to each petri dish and spreads evenly using sterile glass rod. Three replicates were used. Nutrient agar media was used to isolate soil bacteria, Potato dextrose agar amended with 30 ppm. Rose Bengal was used for isolation of soil fungi. Actinomycetes were isolated by using glucose asparagine agar media. Incubation for bacteria was at 27-30 C for 24-48hr, for actinomycetes, and fungi was at 27-30C for 3-5 days. To assess the effect of herbicides on nitrogen fixing bacteria three mature plants were collected and washed in running water and number of nodules per plant was counted. The average number of colony forming units per gram of soil of bacteria, fungi, and actinomycetes of three replicates were recorded using colony counter. And the average number of nodules per plant of three replicates per plot were obtained. The data was

statistically analyzed.

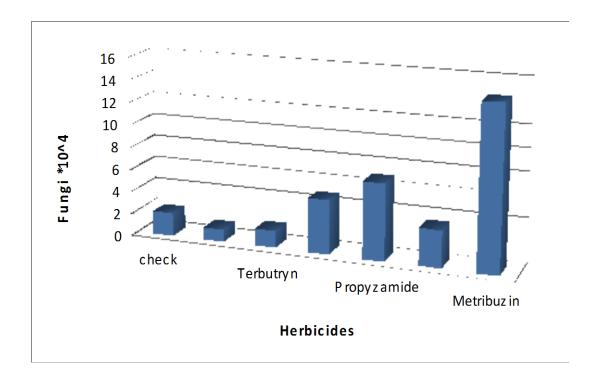
# 3. The results and Discussion



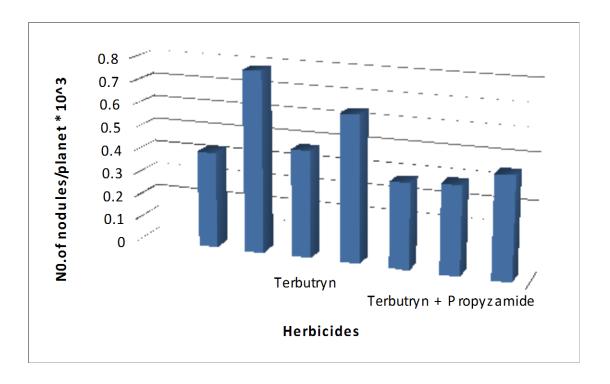
The results of the study table 1 and figure (1) showed Heterotrophic bacteria were reduced by Carbetamide, while Metribuzin increased the bacterial population, other treatments showed no significant effects on number of soil bacteria.



All herbicide treatments used significantly reduced the actinomycetes population of soil figure (2).



All herbicide treatments showed no significant effects on soil fungi except Propyzamide and Metribuzin which showed an increase in total number of soil fungi figure (3).



All herbicides showed no significant effects on the number of nodules per plant except carbetamide and hand weeding that increased total number of nodules per plant compared with the control figure (4).

The results of the study showed similar findings with other studies. The use of herbicides shown to

influence soil microflora and nitrogen fixing bacteria in pea fields and legume plants [5,7,14,17,18]. Herbicides found to stimulate fungal growth [2,5]. Pre -emergent herbicides Atrazine, Metsulfuron methyl ,Metolachlor and Anilofos found to have no negative effects on soil microbial population including soil fungi , bacteria and actinomycetes in rain fed mayze field [22]. In sugar cane premergent herbicides including Atrazine and Amertryn found to have a negative effects on soil microflora including soil fungi ,bacteria, and actinomycetes [28]. The effect of herbicides on soil bacteria found to depend on soil type ,soil moisture, temperature and soil PH and nutrient availability [27]. The lack of inhibitory effect of herbicides on nodulation obtained could be due to their rapid inactivation in soil or their translocation along with Photosynth ate, to distant metabolic sink [9]. Other studies have shown that herbicides had no direct effects on the rhizobium but rather on plant growth [8,13,15,16,23,29]. And as found in our experiment and other studies , herbicides could have inhibitory effects on soil microorganisms ,but generally the microbial populations react by increasing their biomass and activity [24]. The use of herbicides have been shown to effect plant growth and have a negative effect on soil microorganisms growth and metabolism [24,31].

# 4. Conclusion

Herbicides used showed different effects on soil microflora which could influence the microbial balance of soil which plays a major rule on soil fertility and crop yield. Studies should continue to assess the effects of herbicides on soil microflora and nitrogen fixing bacteria on different legume crops in Libya.

Table [1]: Herbicides Effects on Soil Microflora and Nitrogen Fixing Bacteria In Pea Field

Herbicides	Doses kg.a.i./hect.	No. of c.f.u./gm.of soil			3 No. of nodules/plant *10
		6 Bacteria *10	4 Fungi *10	5 Actinomycetes *10	
check		33.6*	2.0*	12.3*	0.413*
Hand weeding		35.6*	1.1*	4.8**	0.776**
Terbutryn	1.5	23.3*	1.5*	7.5**	0.46*
Carbetamide	3.5	16.6**	4.8*	0.3**	0.63**
Propyzamide	0.5	44.0*	6.8**	0.48**	0.366*
Terbutryn + Propyzamide	1.5 + 0.5	20.0*	3.3*	0.53**	0.38*
Metribuzin	0.5	58.0**	14.5**	0.48**	0.44*
L.S.D. at.0.05		16.5	3.92	1.76	0.08

<sup>\*</sup>\_ No significant , \*\*\_ significant difference according to least significant difference

# References

- [1] bd-Alla,M.H., S. A. Omar. 1993. Herbicides Effects on Nodulation, Growth and Nitrogen yield of Faba bean induced by indigenous Rhizobium Leguminosarum. Zentral bdatt fur Mikrobiologie.volume 148:issue 8 p.593-597
- [2] Adil A.Ehussain., Afrah T. Mohamed., Marmar A. Elsddig., Ashraf M. Sharif., and Awad G. Osman. 2012. Effects of Oxyflourfen Herbicide on Microorganisms in Loam and Silt loam soils. Research Journal of Environmental Sciences 6:134-145.
- [3] Ahemad. M., and M.S. Khan 2009 Toxicity Assement of Herbicides Quizalafop-p-ethyl and Clodinafop towards Rhizobium pea Symbiosis . Bull. Environ. Contam. Toxicol. 82:761-66.
- [4] Anderson .,J.A., k.,S.L., Rogers. W.,Bellotte. and G.,Gill. 2004. Influence of Chlorsulfuron on rhizobial growth, nodule formation, and nitrogen fixation with chickpea. Aust. J. Agric. Res. 55:1059-1070
- [5] Bopaiah, B.M., Rai. P.V. 1979. Effect of four common herbicides on the population of microorganisms in Red Sandy Soil. Plant and Soil vol. 52:p. 451-452
- [6] Duah, Yentumi., S., and Johnson. D. B. 1986. Changes in soil micrflora in response to repeated application of some pesticides . Soil biology and Biochemistyr .18-629-635.
- [7] Durgo,K.,M.,Beerna.,S., and Abraham.C.,T., 2008. Effect of 2-4-D.residues on soil miroflora. Journal of Tropical Agriculture.46 [1-2] 76-78
- [8] Eberbach, P.L., and L.A. Douglas. 1991. Effect of herbicide residues in a sandy loam on the growth, nodulation and nitrogenase activity [C2H2/C2H4] of *Trifolium subterraneum*. Plant Soil 131:67-76.
- [9] Fernandez Pascual. M., Delernzoc, Pozuelo. J.M., Defelipe, M.R. 1992. Alteration induced by four herbicides on Lupin nodule cortex structure, protein metabolism and some senescence related enzymes. J. Plant Physiol. 140 [385-390]
- [10] Flores, M., and M. Barbachano. 1992. Effects of herbicides Gramoxone, Diuron and Totacol® on growth and nodulation of three strains of *Rhizobium meliloti*. Sci. Total Environ. 123/124:249-260.
- [11] Fox, J.E., M. Starcevic, K.Y. Kow, M.E. Burow, and J.A. McLachlan. 2001. Endocrine disrupters and flavonoid signaling. Nature 413:128-129
- [12] Fox, J.E., M. Starcevic, P.E. Jones, M.E. Burow, and J.A. McLachlan. 2004. Phytoestrogen signaling and symbiotic gene activation are disrupted by endocrine-disrupting chemicals. Environ. Health Perspect. 112:672-677
- [13] Gonzalez, A., C. Gonzalez-Murua, and M. Royuela. 1996. Influence of imazethapyr on

- Rhizobium growth and its symbiosis with pea [Pisum sativum]. Weed Sci. 44:31-37.
- [14] Jensen,P J.993.Tolerance to foliage applied herbicides in combining peas: Effect of growth stage m cultivar type and herbicide. Crop Prot. 12:214-218.
- [15] Khan, M.S., A. Zaidi, and M. Aamil. 2004. Influence of herbicides on chickpea Mesorhizobium symbiosis. Agronomie 24:123-1 27
- [16] Khan, M.S., A. Zaidi, and P.Q. Rizvi. 2006. Biotoxic effects of herbicides on growth, nodulation, nitrogenase activity, and seed production in chickpeas. Commun. Soil Sci. Plant Anal. 37:1783-1793
- [17] koopman, D.J., P.G. Tow, T.G. Reeves, and A.H. Gibson. 1995. Soil acidification, chlorsulfuron application and *Rhizobium meliloti* as factors in lucerne yield decline. Soil Biol. Biochem. 27:673-677. nviron. 123/124:249-260
- [18] Ledgard, S.F., and K.W. Steele. 1992. Biological nitrogen fixation in mixed legume/grass pastures. Plant Soil 141:137-153
- [19] Mallik, M., and K. Tesfai. 1985. Pesticidal effect on soybean-*rhizobia* symbiosis. Plant Soil 85:33-41.
- [20] Michiels, J., C. Verreth, and J. Vanderleg. 1994. Effects of temperature stress on bean nodulating rhizobium strains. Appl. Environ. Microbiol. 60:12061212.
- [21] Moorman, T.B., J.M. Becerril, J. Lydon, and S. O'Duke. 1992. Production of hydroxybenzoic acids by *Bradyrhizobium japonicum* strains after treatment with glyphosate. J. Agric. Food Chem. 40:289-293
- [22] Ramesh G., nadanassababady T. 2005. Impact of herbicides on weeds and soil ecosystem of rainfed maize [Zea mays l. ] . Indian J. of Agric, Research . volume :39,Issue:1pp31-36
- [23] Rennie, R.J., and S. Dubetz. 1984. Effect of fungicides and herbicides on nodulation and N2-fixation in soybean fields lacking indigenous *Rhizobium japonicum*. Agron. J. 76:451-454.
- [24] Sawick., A., Skrzypczak., G., Blecharczyk., A. 1996 Influence of Imazethapy and Linuron on soil microorganisms under legume crops. Proceeding of The scond International Weed Control Congress. Copenhagen. vol. 1.361.
- [25] Singh, G., and D. Wright. 1999. Effects of herbicides on nodulation, symbiotic nitrogen fixation, growth and yield of pea [*Pisum sativum*]. J. Agric. Sci. 133:21-30
- [26] Singh, G., and D. Wright. 2002. *In vitro* studies on the effects of herbicides on the growth of rhizobia. Lett. Appl. Microbiol. 35:12-16.
- [27] Smith A. E. 1982. Herbicides and The soil environment In Canada. Canadian Journal of Soil

Science. 62:433-460

- [28] Soumen Bera, Ratikanta Ghosh . 2013. Soil Microflora and weed management as influenced bt Atrazin 50% wp in Sugar Cane. Universal Journal of Agriculture Research 1[2] 41-47
- [29] Sprout, S. L., L.M. Nelson, and J.J. Germida. 1992. Influence of metribuzin on the *Rhizobium leguminosarum* lentil [*Lens culinaris*] symbiosis. Can. J. Microbiol. 38:343-349. . vol.Biotec1.36
- [30] Peoples M. B., J.K.Ladha and D.F.Herridge 1995 Enhancing Legum N Fixation Through Plant and Soil Management . Plant Soil 174:83-101
- [31] Quinn, J.P., Peden, J.M., Dick. R.E., 1988. Glyphosate Tolerance and Utiliztaion by the microflora of soil. Appl Microbiol, biotechnol. 29,511.
- [32] Tate R.L. 1995. Soil Microbiology [ Symbiotic Nitrogen Fixation ] p.307-333. Jhon Wiley and Sons Inc. New York NY.