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EVALUATION OF ORGANIC FUNGICIDES FOR CONTROL OF BLACK POINT IN DURUM WHEAT

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ABSTRACT

The organic fungicides Cu ProtectiveR, PHC MilStop PlusR, CYR MEGAR, and FulciteR were evaluated for control of black point of wheat at the Norman E. Borlaug Experimental Station in the Yaqui Valley, Sonora, Mexico, during the crop season 2020-2021. Durum wheat commercial cultivar CIRNO C2008 was used for this evaluation by sowing on December 1, 2020, in an organic certified plot with a seed density of 70 kg ha-1. Four complementary irrigations were applied in addition to the irrigation for seed germination. Weed control was done manually. The organic fungicides were applied 83 and 111 days after sowing (grain filling and physiological maturity, respectively) with a Swissmex back pack sprayer (10 liter capacity) at the rates of 2.0, 1.5, and 6.0 liters of commercial product, as well as 7.0 grams, respectively for a volume of 300 L of water. The experimental design was a randomized complete block with three replications, the experimental plot consisted of three beds with double row 5 m long, and beds separated by 0.80 m, and the experimental unit consisted of one bed with two rows 1 m long. Harvest was done manually with a sickle and threshing with a Pullman stationary machine. The percentage of infected grains was calculated by counting infected and healthy grain from a sample of 100 from each experimental unit. The ANOVA was performed with the MSTAT version 2.10 and the mean comparison with the LSD test (p= 0.05). The average percentage of infected grains was 3.33, 4.33, 4.67, 9.67, and 16.25% for Cu ProtectiveR, PHC MilStop PlusR, CYR MEGAR, FulciteR, and the untreated check, respectively. The first three were statistically similar, different to FulciteR and the untreated check, and FulciteR was different than the untreated check. The biological effectiveness of Cu ProtectiveR, PHC MilStop PlusR, CYR MEGAR, and FulciteR for control of black point of wheat was 79.4, 73.3, 71.2, and 40.5%, respectively.

Keywords: Triticum durum, durum wheat, black point, Alternaria spp., organic fungicides.

1. INTRODUCTION

Wheat is one of the most important cereals for human consumption and it is the second most important in worldwide production (FAOSTAT, 2021; Ramos, 2013). This cereal is cultivated under diverse climatic conditions and it has the widest adaptation of all the cereal crop species (Briggle and Curtis, 1987). Wheat has been a staple food for civilizations from Europe, Asia, and North Africa for more than 8,000 years (FAO, 1999). In Mexico it is an important crop for consumption and the economic revenues generated by the production. The most important wheat-producing states are Sonora, Guanajuato, Sinaloa, and Baja California. In 2020, 230,087.29 ha were sown with wheat in Sonora with a harvest of 1,532,757.29 t (SIAP, 2022). In southern Sonora durum wheat (*Triticum durum* Desf.) has three sale paths: for animal feed, for the industry, and for export. The industry requires high quality standards and therefore a premium price is granted when the wheat grain complies with this feature, otherwise, the buyer will impose a penalty (Garza and Taddie, 2016), such as wheat grain affected with black point (BP) (*Alternaria* spp.), and although this disease does not affect yield and protein value, it does affect commercialization due to the blackening of the embryo area which affects the flour quality (Chávez and Kohli, 2013). The flour and pasta obtained from infected wheat grain frequently present unpleasant color and odor (Rodríguez *et al*., 2009). Perelló *et al*. (2015) reported that the increase in the amount of BP during milling, causes deterioration on the pasta visual quality, and affects the yield of semolina of durum wheat (García *et al*., 2012). In countries such as Canada, the United States of America, and Australia, penalties are imposed when the grain present a percentage of BP above 10, 2, and 5% in the case of soft wheat, and 5, 2, and 5% for durum wheat, respectively (Australian Wheat Board, 2000, cited by García *et al*., 2012). In Paraguay measures are stricter, since they accept a maximum between 0.1 and 0.3% of grain with BP (Chávez and Kohli, 2013). No official data were found related to tolerance levels allowed/penalties for BP in wheat in Mexico. *Alternaria* spp., *Fusarium* spp., and *Helminthosporium* spp. are among many fungi that can be found in newly harvested wheat grain (*Triticum* spp.), particularly when relative humidity exceeds 90% and seed moisture content 20% (Mathur and Cunfer, 1993). Black point or kernel smudge is favored by rainfall during seed maturity and humid weather prevailing before harvest (Prescott *et al*., 1986). BP also causes premature seed senescence because many of the fungi are saprophytic (Wiese, 1987). *Bipolaris sorokiniana* (Sacc.) Shoemaker and *Alternaria alternata* (Fr.:Fr.) Keissl. are considered the primary agents of the disease (Mathur and Cunfer, 1993). Although infected spikers may look normal, they may have elliptical, brown to dark brown lesions on the glume inner side. Brown to dark brown or blackish localized areas, may be present around the embryo end of seeds (Figure 1) (Adlakha and Joshi, 1974; Hanson and Christensen, 1953; Rana and Gupta, 1982; cited by Mathur and Cunfer, 1993; García *et al*., 2012). Severe infection causes discoloration and shrivelling of the whole seed (Adlakha and Joshi, 1974).

Figure 1. Symptoms of black point in wheat grain. The grain on the left shows the general effect of the disease on the pericarp; the inside (starch granules) remain intact.

Although BP is an endemic disease of durum (*Triticum durum* Desf.) and bread wheat (*Triticum aestivum* L.), in southern Sonora, Mexico (Fuentes-Dávila *et al*., 2014b), incidence varies every crop season. This disease also affects triticale (X *Triticosecale* Wittmack) (Fuentes-Dávila *et al*., 2014a; Wiese, 1987). Several reports indicate that the susceptibility of cultivars and the irrigation management might be the key factors to control the disease (Rodríguez *et al*., 2009; García *et al*., 2012; Prioletta, 2015). Research has been done testing a wide variety of chemical fungicides for the control of phytopathogens of the wheat grain (Mellado *et al*., 1990; Da Ros *et al*., 2018; Mariscal-Amaro *et al*., 2020); however, the continuous use of this type of synthetic products under the conventional agriculture will have greater negative effects on global ecosystems (Rodríguez, 2018). Therefore, there is an increase of farmers and researchers who are looking for ecological and natural alternatives, with the objective to obtain healthy food free of toxicity and eliminate the use of agrochemical products (Meneses, 2017). The objective of this work was to evaluate the biological effectiveness of organic fungicides for control of BP in wheat in a plot with organic certification.

2. MATERIALS AND METHODS

The evaluation was carried out at the Norman E. Borlaug Experimental Station in the Yaqui Valley, Sonora, Mexico, during the crop season fall-winter 2020-2021, in a plot with clay soil and with organic certification (AGRICERT MEXICO–SENASICA, 2019; BIOAGRICERT, 2019a, b). Durum wheat cultivar CIRNO C2008 (Figueroa-López *et al*., 2010) was used for the evaluation; the land was prepared with three passes of a harrow and fertilization was done with 9 t ha^{-1} of chicken manure, broadcasted and incorporated into the soil during the third harrow pass. Sowing was done on December 1, 2020, with a seed density of 70 kg ha^{-1}. Four complementary irrigations were applied in addition to the irrigation for seed germination. Weed control was done manually three times. The organic fungicides with OMRI registration (Organic Materials Review Institute, https://www.omri.org/) Cu Protective^R, PHC MilStop Plus^R, CYR MEGA^R, and Fulcite^R were applied 83 and 111 days after sowing (grain filling and physiological maturity, respectively) with a Swissmex back pack sprayer (10 liter capacity) at the rates of 2.0, 1.5, and 6.0 liters of commercial product, as well as 7.0 grams, respectively, for a volume of 300 L of water (Table 1). CYR MEGA is a botanic bactericide and fungicide which contains 95% of creosote bush extract (*Larrea tridentata* (Seesé and Moc. ex D.C.) Coville) (CYR Agroquímica, 2018). The active ingredient of PHC MilStop Plus^R is potassium bicarbonate (85%) specially formulated for agricultural use; it is an organic contact fungicide with wide spectrum on fungal diseases of plants (PHC México, 2022). Cu Protective contains 19% of copper sulphate, 81% of *Aloe vera* (L.) Burm. f. extract, and organic conditioner (SMART Agrosolutions, 2022). Fulcite^R contains 60% of the bacterium *Streptomyces* spp., 1% glucosamine, 3% organic matter, 0.65% humic acid, 0.15% fulvic acid, and 35.2% thinners and conditioners (Agrokorita, 2017; NAY-CHEM, 2017). The experimental design was a randomized complete block with three replications; the experimental plot consisted of three beds with double row 5 m long, and beds separated by 0.80 m, and the experimental unit consisted of one bed with two rows 1 m long. Harvest was done manually with a sickle and threshing with a Pullman stationary machine. The percentage of infected grains was calculated by counting infected and healthy grains from a sample of 100 from each experimental unit. The ANOVA was performed with the MSTAT version 2.10 and the mean comparison with the LSD test (p= 0.05). The biological effectiveness of the products was calculated by Abbot´s formula (1925). A kilogram of the grain obtained was sent to the diagnostic laboratory of the Council for Agriculture Investigation and Experimentation of the state of Sonora (PIEAES, 2021), in order to identify the fungi present in grains with BP.

Table 1. Commercial names of the organic fungicides evaluated, their active ingredient and the rate applied on durum wheat commercial cultivar CIRNO C2008 for control of black point, at the Norman E. Borlaug Experimental Station in the Yaqui Valley, Sonora, Mexico, during the crop season 2020-2021.

Treatment	Active ingredient and concentration	Rate* (ha^{-1})
CYR MEGA^R	*Larrea tridentata* extract (95%)	6.0 L
PHC MilStop Plus^R	Potassium bicarbonate (85%)	1.5 L
Cu Protective^R	Copper sulphate (19%)	2.0 L
Fulcite^R	Bacteria *Streptomyces* spp. (60%)	7.0 g
Untreated check		

*Commercial rates of products in liters and grams (Fulcite^R).

3. RESULTS AND DISCUSSION

The report issued by the diagnostic laboratory indicated a percentage of 6.75 of grains with the presence of *Alternaria* spp. (PIEAES, 2021), which agrees with the report by Cortés *et al*. (2019), where they evaluated bread wheat cultivars RSI-Norman F-2008 and Borlaug 100 which showed 2 and 7.8% average percentage of infected grains, respectively. Significant statistical differences were found between treatments (Table 2) ($p \leq 0.05$), and all treatments showed the presence of grains affected by BP. The range of infected grains from plots treated with the organic fungicide Cu Protective^R was 2 to 5, 2 to 6 for PHC MilStop Plus^{R,} 2 to 9 for CYR MEGA, 5 to 12 for Fulcite^R, and 15.8 to 17 for the untreated check. Although control of the disease was not achieved satisfactorily, the biological effectiveness of Cu Protective^R, PHC MilStop Plus^R, CYR MEGA^R, and Fulcite^R for control of BP of wheat was 79.4, 73.3, 71.2, and 40.5%, respectively. Cu Protective^R had the highest effect on the disease with a difference of 12.92% with respect to the untreated check. Cupper salt is a substance allowed for organic agriculture (SENASICA, 2020) which is one of the ingredients of the Bordeaux mixture, the first man-made fungicide for control of plant diseases; its wide spectrum of action as an effective bactericide and fungicide, as well as its low cost, has kept it in the market nowadays (Broome and Donaldson, 2010; Cruz, 2004; Agrios, 2005).

Potassium bicarbonate is another product which has shown control of diseases in different crops, such as in cucumber (*Cucumis sativus* L.) for control of powdery mildew (*Oidium* sp.) (Yañez *et al*., 2012; 2014), and for powdery mildew (*Sphaerotheca mors-uvae* (Schwein.) Berk. & M.A. Curtis) of gooseberry (*Ribes uva-crispa* L.) (Wenneker and Kanne, 2010), and in this case, a difference of 11.92% in the average of infected grains was obtained in relation to the untreated check. The active ingredient of CYR MEGA[R] is the extract of *Larrea tridentata* (Creosote bush), a perennial shrub from the Chihuahuan, Sonoran and Mohave deserts of North America. The leaves contain a thick resin, which behaves as a barrier that diminishes transpiration, and secondary metabolites like phenols, lignans and flavonoids, appear to be biochemical defenses that repel the aggression of herbivores, fungi, and other microorganisms, since there are not known pests, diseases, or animals that attack this plant (Lira-Saldívar, 2003).

Table 2. Mean separation by LSD test of the transformed percentages of infected grain with black point, treated with organic fungicides on durum wheat cultivar CIRNO C2008, at the Norman E. Borlaug Experimental Station in the Yaqui Valley, Sonora, Mexico, during the crop season 2020-2021.

Treatment	Infected grain		Mean separation
	Real	Transformed	
Cu Protective[R]	3.33	10.33	A
PHC MilStop Plus[R]	4.33	11.73	A
CYR MEGA[R]	4.67	11,87	A
Fulcite[R]	9.67	17.83	B
Untreated check	16.25	23.80	C

LSD = 4.6 (p= 0.05). Treatments with the same letter indicate that there are no statistical significant differences.

L. tridentata has antifungal activity *in vitro* on at least 17 fungal pathogens of economic importance (Lira-Saldívar, 2003; Lira-Saldívar *et al*., 2003; López-Benítez *et al*., 2005; Peñuelas-Rubio *et al*., 2017); likewise, extracts and ground plant material incorporated into the soil as powder inhibit or control *in vivo* six fungi that affect agricultural crops. Some studies also have reported the nematicidal or nematostatic effect of *L. tridentata* against nine genera of nematodes, and to act as repellent against one insect (Lira-Saldívar, 2003). On the other hand, there have been bioassays with microorganisms that attack humans, indicating that more than 45 bacteria, 10 yeast, nine fungi and three intestinal parasites are susceptible to *L. tridentata* resin or its constituents (Lira-Saldívar, 2003). The antiviral effect of creosote bush also has been documented, demonstrating that flavonoids are active against virus that affect RNA, and that cause severe diseases like polio, AIDS and herpes (Lira-Saldívar, 2003). *L. tridentata* has also shown antibacterial activity against *Clavibacter michiganensis* sbsp. *Michiganensis* (Smith) Davis *et al*., *Pseudomonas syringae* van Hall, and *Xanthomonas campestris* (Pammel) Dowson (Morales-Ubaldo *et al*., 2021). In our study, a difference of 11.58% in the average of infected grains was obtained in relation to the untreated check. Bacteria are used in the industry in ways that exploit their natural metabolic capabilities. They are used in manufacture of foods,

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antibiotics, probiotics, drugs, vaccines, starter cultures, insecticides, enzymes, fuels and solvents. Also, with genetic engineering technology, bacteria can be programmed to make various substances used in food science, agriculture and medicine. The genetic systems of bacteria are the foundation of the biotechnology industry (Todar, 2022). Using *Agrobacterium tumefaciens* (Smith and Townsend) Cohn, plants have been genetically engineered conferring resistance to certain pests, herbicides, and phytopathogens (Babalola, 2010; Recep *et al*., 2009). *Bacillus* spp. can be used to induced systemic resistance in plants (Choudhary and Johri, 2009), there are plant- growth-promoting bacteria (Herschkovitz *et al*., 2005; Latha *et al*., 2009; Principe *et al*., 2007), and beneficial rhizobacteria for legumes (Hynes *et al*., 2008). *Streptomyces* spp., the most abundant actinobacteria in soil, are responsible for the production of many drug molecules and represent a great resource for the discovery of new ones; they are also efficient plant colonizers and able to employ different mechanisms of control against toxigenic fungi on cereals (Colombo *et al*., 2019). Since they constitute a source of antibiotics and bioactive compounds, they are considered of great potential for organic agriculture (Buzón-Durán *et al*., 2020). In this study, a difference of 6.58% in the average of infected grains was obtained in relation to the untreated check. The percentage of infected grains with black point in crop season 2020-2021 at the Norman E. Borlaug Experimental Station was relatively low, since the untreated check with durum wheat cultivar CIRNO C2008 had an average of 16.25 with a range of 3 to 23%. This cultivar has shown to be moderately resistant since during the crop seasons fall-winter 2009-2010 to 2020-2021, with the exception of seasons 2010-2011 and 2019-2020, the average percentage of infected grains with BP was 3.68% with a range of 0.62 to 14.4%, although in 2009-2010 in one sowing date it had 13.93% and in 2017-2018 27.4% (Figure 2) (Fuentes-Dávila *et al*., 2013, 2014c, 2015, 2016, 2018a, 2018b, 2019, 2020a, 2020b, 2021).

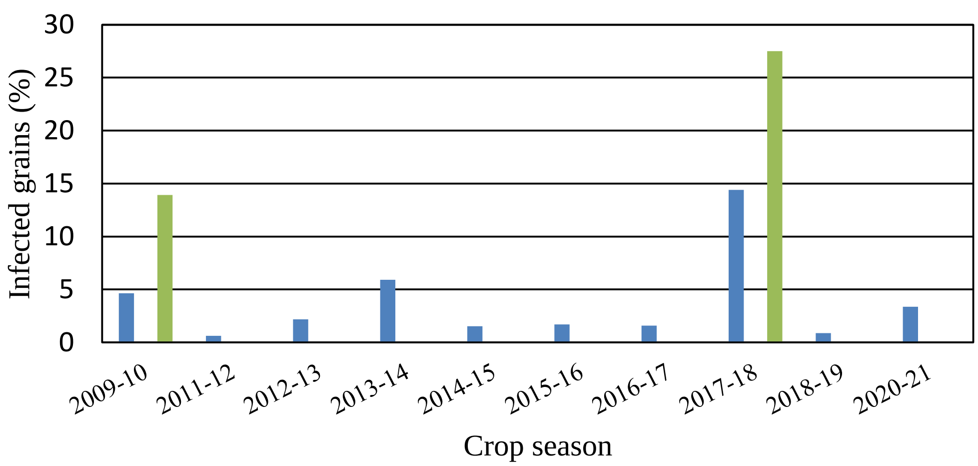

Figure 2. Average percentage (blue bars) of black point in durum wheat cultivar CIRNO C2008 during ten crop seasons, at the Norman E. Borlaug Experimental Station in the Yaqui Valley, Sonora, Mexico. Green bars indicate the highest percentage of grain infected with BP in a particular sowing date in that season.

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In contrast, with other durum genotypes, Fernández *et al*. (1994) reported a 41% average of BP infected grains out of a group of more than 130 genotypes in 1992 in Outlook, Saskatchewan, Canada, and Fuentes-Dávila *et al*. (2015) reported that the genotype CHEN1/TEZ/3/GUIL//CIT71/CII/4/SORA/PLATA_12/5/STOT//ALTAR84/ALD/6/SOMAT_3/PHAX_1//TILO_1/LOTUS_4/7/AJAIA_12/F3LOCAL(SEL.ETHIO.135.85)//PLATA_13/4/CHEN_1/TEZ/3/GUIL//CIT71/CII/5/SORA/2*PLATA_12//SOMAT_3 showed 26.22% infected grains. All the organic fungicides evaluated in this study were able to reduce the percentage of infected grains with BP, but an acceptable level of control would be greater than 85% biological effectiveness; therefore, it is necessary of evaluate other rates as well as different number of application of the products.

4. CONCLUSION

The biological effectiveness of the organic fungicides Cu Protective^{R}, PHC MilStop Plus^{R}, CYR MEGA^{R}, and Fulcite^{R} for control of black point in durum wheat cultivar CIRNO C2008 was 79.4, 73.3, 71.2, and 40.5%, respectively.

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