

EFFECT OF AQUEOUS EXTRACT OF *CARTHAMUS TINCTORIUS* L. ONGERMINATION AND INITIAL SEEDLING GROWTH OF *ORYZA PUNCTATA* L.

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ABSTRACT

Chemical weed control method may cause environmental hazards and residual effects in crops and soil. Alternate approach to control weeds is getting attentions in sustainable production system. Use of aqueous extracts of crops is getting scientific attention as eco-friendly alternative to synthetic herbicides especially under scenario of fast increasing herbicide resistance in weeds. To investigate the herbicidal potential of aqueous extract of winter crop Carthamus tinctorius L. (safflower) on summer weed Oryza punctata L. (red rice) seed emergence and initial seedling growth, a study was planned. In this study, seeds of Oryza punctata were incubated in seven concentrations (0, 0.25, 0.50, 1, 2, 4, and 8%) of different parts i.e. leaves, stem and fruit of C. tinctorius. All the tested concentrations of various plant parts of C. tinctorius significantly inhibited the mean emergence time, emergence index, emergence percentage (%), time taken to 50% emergence as well as growth of O. punctata weed. However, maximum mean emergence time (4.73 days), time taken to 50% emergence (4.19 days) was noted at 8% concentration and emergence index of O. punctata was noted at 0.25% concentration when fruit extract was applied. Leaf aqueous extract at the concentration of 8% caused the lowest root length (1.53 cm), shoot length (4.51 cm) and fresh weight (54.72 g) of O. punctata. Results suggested leaf and flower extract of C. tinctorius at 8% concentration can be used potential bio-herbicide for the control of red rice.

Keywords: Aqueous extract, seedling growth, inhibitory, weed growth, plant parts, promotor.

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INTRODUCTION

The use of herbicide in controlling weed is one of the major weed control strategy and is considered as the time saving, most effective and economical method to control the targeted weeds (Kraehmer *et al.*, 2014). However, this excessive use of herbicides in weeds management causes serious threat to both environment and public health (Asghari and Tewari, 2007). Furthermore, increasing herbicide resistance in weeds have made chemical weeds control an unsuitable method to sustain crop production (Abbas *et al.*, 2016a); allelopathy is quite effective to control resistant weeds (Abbas *et al.*, 2016b). Therefore, there is necessity to develop an alternate weed control method which are less or no dependent on synthetic herbicides and less toxic to environment (Singh *et al.*, 2011). Weed crop competition is one of the major biotic constraints in the production of rice. In rice 12% reduction in the yield is due to the weeds (Singh *et al.*, 2011). Weeds crop competition is higher in early growth stages and un-control of weeds during early growth stages of crop result in poor crop growth of and decrease in grain yield (Fahad *et al.*, 2015). Biological control of weeds is an important method of weed control and is also environment friendly and very cheap (Abbas *et al.*, 2016b). Grassy and broad-leaf plants have the potential to suppress weed (Anjum *et al.*, 2005; Javaid and Khna, 2020; Javaid *et al.*, 2020). Allelopathic potential of safflower has been reported in several studies. Miri (2011) reported that safflower significantly inhibit the germination and root and shoot growth of wild barley (*Hordeum spontaneum* L.) and has great potential for management of this weed in wheat (*Triticum aestivum* L.) production. Farhodi and Lee (2012) showed that safflower extracts inhibited the induction of α -amylase in wild mustard (*Sinapis arvensis* L.) seeds. Safflower has also allelopathic influence on the barley seed germination and result in less seed germination and poor growth of seedling (Ashrafi *et al.*, 2008). Furthermore,

Bonamigo *et al.*, (2013) demonstrated that seedling emergence and early growth stages of canola (*Brassica napus* L.) were negatively affected by safflower aqueous extracts. Little is known about safflower allelopathic potential. The leaves and roots of sunflower are key source of allelochemicals (Yanget *et al.*, 2007). The chemicals like phenolics, flavonoids or terpenoids are generally recognized to own allelopathic characteristics (Jabran *et al.*, 2015).

Therefore, the need of this experiment was to investigate the allelopathic potential of *C. tinctorius* plant parts such as leaf, stem and fruit on germination and initial seedling growth of *O. punctata*.

MATERIALS AND METHODS

Collection of *C. tinctorius* plant parts

To make aqueous extract *C. tinctorius* plants were collected from Agronomic Farm, University of Agriculture Faisalabad. and harvested above the ground surface at physiological maturity and dried for two weeks at ambient temperature. After drying, different parts of plants were separate and chopped into 2 cm pieces for extract formation.

Preparation of *C. tinctorius* Aqueous Extracts

C. tinctorius aqueous extracts of various parts were made by adding 10 g of chopped dried plant material into 100mL of distilled water in bottles separately at ratio of 1:10 w/v. At room temperature plant material were soaked in the water for at least 24 hours. These aqueous extracts were made from each desired part of *C. tinctorius* such as leaves, stem and fruit then the material was passed through a cheese cotton cloth to attain the 10 % water extracts of different parts of *C. tinctorius* which was used as stock solution. The stock solution diluted to prepare the required concentration of 0.25%, 0.5%, 1%, 2%, 4% and 8%.

Laboratory experiment

Each dilution of each extract placed in separate bottles and then tagged these

bottles by name of each dilution with its plant name too carefully for their easy utilization in next procedure. The experiment was conducted in each 9cm petri plate lined with filter no.10-filter paper.

To estimate the allelopathic effect 0%, 0.25%, 0.5%, 1%, 2%, 4% and 8% concentration of each plant part of *C. tinctorius* were applied on *O. punctata* seeds separately. A 20 seeds of *O. punctata* were placed in each Petri plates containing filter paper. A 7mL of all *C. tinctorius* extracts dilutions of each part (leaves, stem, flower and fruit) was added in respective petri plates having 3 replications of each dilution. One treatment was kept as control and moist with distilled water. To minimize the excess of evaporation petri plates were covered and rapped with parafilm. The petri plates were kept at the temperature of 30°C and were again moistened with 3 mL after one week. The data regarding emergence of the seeds were noted every day for 14 days. After the 14 days, harvest the germinated seedlings of *O. punctata* and observed the different parameters like shoot length, root length, fresh and dry weight. Fresh weight was recorded instantly after harvesting while the dry weight of seedling was observed after oven drying for two days at 60 °C.

Data collection

Mean emergence time of *O. punctata* (day)

Ellis and Reberts (1981) equation were used to examine the mean emergence time (MET).

$$MET = \frac{\sum(Dn)}{\sum n}$$

Emergence index of *O. punctata*

By using formula of association of the official seed analysis (1983) we record the emergence index

$$GI = \frac{\text{No. of emerged seeds}}{\text{Days of first count}} + \dots + \frac{\text{No. of emerged seeds}}{\text{Days of final count}}$$

Emergence percentage of *O. punctata* (%)

No of emerged seeds were counted daily according to the method of the association of Official Seed Analysis (1990) and converted into emergence percentage by the following formula.

$$\text{Emergence (\%)} = \frac{\text{No. of emerged seeds}}{\text{Total seeds}} \times 100$$

Time taken to 50% emergence of *O. punctata* (day)

The time to the 50% emergence (E_{50}) was recorded by using the formula purposed by Coolbear *et al.* (1984)

$$E50 = t_i + \left[\frac{\frac{N}{2} - n_i}{n_j - n_i} \right] (t_j - t_i)$$

Growth attributes of *O. punctata*

All seedlings from each petri plate were separate 14 days after emergence. After that both shoot length and root length were calculated by using meter rod from base level to top of the plants. Seedlings fresh weight was examined by separating seedlings from petri dish and measuring by using digital weight balance. Seedlings dry weight was calculated by oven drying the seedlings for 48 hours at 60 °C then weighted to get average dry weight of seedling by using digital balance.

Statistical analysis

Statistics software (version, 8.1Statistix, Tallahassee, FL, USA) was used to analyses the collected data and least significant difference test (LSD) was used to compare the means of treatment at probability level of 5%.

RESULTS AND DISCUSSIONS

Mean emergence time of *O. punctata* (days)

Aqueous extract of *C. tinctorius* various parts produced non-significant effect on mean emergence time of *O. punctata* at all tested concentrations (Table 1). The highest mean emergence time (4.54 days) was observed at 2% concentration while lowest mean emergence time (4.29 days) was noted when distilled water applied (0% concentration). The interactive effect of different concentration and plants parts displayed that maximum emergence time (4.73 days) was examined under flower extract at 8% concentration. The stimulatory effect of aqueous extracts of *C. tinctorius* plant parts and their different concentrations on mean germination time

may be due to presence of allelochemicals that produce hermetic effect at its lower concentration on *O. punctata* germination. Our study is contradicted with the findings of Rose and Anitha (2012) who found that water extracts of *Euphorbia hirta* at different concentrations showed significant inhibitory impact on the groundnut mean emergence time and this inhibitory effect was directly proportional to the concentration of extracts.

Emergence index of *O. punctata*

The influence of different concentration of *C. tinctorius* extracts was found non-significant on emergence index of *O. punctata* (Table 2). The highest value of emergence index (3.62) of *O. punctata* seedling was obtained at 2% concentration while lowest emergence index (3.12) was observed at 4% concentration. Different plant parts of *C. tinctorius* were also non-significant on emergence index of *O. punctata* seedlings. However, the stem showed the stimulatory effect on emergence index of *O. punctata* seedlings whereas, leaves and flower extracts showed somewhat inhibitory response on *O. punctata* seedlings. The interaction effect of plant parts and their various concentrations was also found non-significant. The maximum emergence index of *O. punctata* was noted at 0.25% concentration under flower extract. Tanveer *et al.*, (2010) revealed that the application *Euphorbia helioscopia* extracts decreased the germination index of *Triticum aestivum* L., *Cicer arietinum* L. and *Lens culinaris* Medic. More delayed germination (higher MGT) of rice with higher (5%) than lower (2.5%) concentration of aqueous extract of *Vicia sativa* has also been reported by previous researchers (Zohaib *et al.*, 2014).

Emergence percentage of *O. punctata* (%)

Allelopathic effect of various concentrations of *C. tinctorius* on emergence of *O. punctata* seedlings was found significant (Table 3). The emergence of *O. punctata* seedlings were inhibited by all the extract's concentration of *C. tinctorius* except at 1 and 2%

concentrations. The maximum emergence percentage was recorded at 1 and 2% concentrations and least emergence percentage (84.44%) was observed at 8% concentrations. Among different plant parts as compared to stem and flower the leaf extract showed more inhibitory effect on emergence percentage, while they all were showed the highest values of emergence percentage. Khan *et al.*, (2011) study allelopathic effect by performing research trial to observe the impact of *S. marianum* aqueous extracts on *Glycine max*, *P. vulgaris*, *C. arietinum*, and *V. radiata* germination. By the influence of extracts of test species significantly decreased the germination index as compared to the control and increase in inhibitory effect was observed by enhancing the extracts concentration. Similarly, Takao *et al.*, (2011) performed a study to observe the influence of *Ipomoea cairica* aqueous extract on the *Bidens Pilosa*, *E. cruss-galli*, *E. heterophylla* and *I. grandifolia*. Results reflect that the test species indicated a significant inhibitory impact on emergence (%) at higher concentrations.

Time taken to 50% emergence of *O. punctata* (days)

Data portrayed in table 4 showed that there were non-significant impact of *C. tinctorius* concentrations on time to 50% emergence of *O. punctata*. However, among all the tested concentrations of *C. tinctorius* maximum (3.59 days) time to complete 50% emergence of *O. punctata* was noted with 8% concentration while the least (3.30 days) was time taken to 50% emergence was recorded under 0% concentration. Among various plant parts of *C. tinctorius* fruit and leaf showed the highest (3.50 days) values of time to 50% emergence of *O. punctata* seedlings as well as stem gave the lowest (3.38 days) value of time to 50% emergence. Interaction effect of various plant parts and their concentration was found significant. Flower showed the stimulatory effect at lower concentrations. While, 8% concentrations caused delayed (4.19 days) emergence. Stem extract also showed significant inhibitory effect at

higher concentrations. According to Anjum and Bajwa (2005), the allelopathic impact of *R. dentatus* aqueous extracts on time to complete the 50% germination of *T. aestivum* and *Helianthus annuus* seedlings was significant. Result reveals that the aqueous extracts of *R. dentatus*, increase in time to complete 50% germination of *T. aestivum* and *Helianthus annuus* seedlings at higher concentration than low concentration.

Shoot length of *O. punctata* seedlings (cm)

The aqueous extracts of *C. tinctorius* produced significant influence on the shoot length of *O. punctata* seedlings (Table 5). Data indicated that substantial response was noted with different concentrations of aqueous extracts and highest (8.27 cm) shoot length of *O. punctata* was measured at 1% concentration. However, an increase in the shoot length of *O. punctata* was observed from 0 to 1% concentrations then a decline was observed from 2 to 8% concentrations of *C. tinctorius* extracts. In case of different plant parts, lowest (5.82 cm) shoot length of *O. punctata* was noted with the aqueous extracts of *C. tinctorius* leaves whereas longest (9.06 cm) shoot lengths were observed with the foliar application of stem extracts of *C. tinctorius*. The interaction among different concentration and plant parts was also significant. It is recorded that at 2% concentration with fruit extract of *C. tinctorius* produced the longest (7.91 cm) shoot length of *O. punctata*. The higher concentration (8%) with aqueous extracts of *C. tinctorius* leaves gave the lowest (4.51 cm) shoot length of *O. punctata*. The shoot length was decreased by the inhibitory influence of safflower water extracts has also been reported by the verdicts of (Khaliq *et al.*, 2009). Mubarak *et al.*, (2011) also supported that the differences in allelopathic potential of various plant parts were significant and extreme values of shoot length were recorded with stem.

Root length of *O. punctata* seedlings (cm)

Allelopathic influence of various *C. tinctorius* concentrations on the root length of *O. punctata* seedlings was significant (Table 6). The root length (cm) of *O. punctata* seedlings was inhibited by all the extract's concentration of *C. tinctorius*. The maximum (3.54 cm) root length was recorded at 0.25% concentration and least root length (2.38 cm) was observed at 8% concentrations. As compared to stem and flower the leaf extract displayed more inhibitory effect on shoot length and produced 1.91 cm shoot length of *O. punctata* (Table 6). However, a significant difference was noted among plant parts and their concentrations and produced greater root length (5.19 cm) at 0.25% concentration under stem extract. The least root length (1.53 cm) was recorded at 8% concentration under leaf extract. The length of root was inhibited by the inhibitory influence of safflower water extracts has also been reported by the verdicts of (Khaliq *et al.*, 2009). The delayed germination and slow seedlings growth can be attributed to the reduction in root length. The significant modifications were detected between water extract of different plant parts concerning root length. Mubarak *et al.*, (2011) also noted differences in allelopathic potential of different plant parts were significant and extreme values of root length were recorded with stem.

Fresh weight(g) of *O. punctata* seedlings(g)

Data depicted in table 7 exhibited that there were non-significant impact of *C. tinctorius* concentrations on fresh weight of *O. punctata* (Table 7). However, among all the tested concentrations of *C. tinctorius* maximum (100.33 g) fresh weight of *O. punctata* was recorded with 0.5% concentration while the least (68.72 g) was observed with 8% concentration. Among various plant parts of *C. tinctorius* flower and stem showed the greater fresh weight of *O. punctata* seedlings as well as leaf gave the lowest (80.37 g) value of fresh weight (Table 7). Interaction effect of various plant parts and their concentration was found significant. The leaf and stem inhibitory effect at all

concentrations. The highest (118.0 g) fresh weight was observed at 4% concentrations under stem extract while the leaf extract under 8% concentration gave the minimum (54.72 g) *O. punctata* fresh weight. As the seedling's growth was inhibited by the inhibitory influence of safflower water extracts, fresh weight also reduced has also been reported by the verdicts of (Khaliq *et al.*, 2009). Mubarak *et al.*, (2011) also supported that the differences in allelopathic potential of various plant parts were non-significant and extreme values of fresh weight were recorded with stem as seedlings grow more on stem extract application than other opium plant parts extracts.

Dry weight(g) of *O. punctata* seedlings(g)

The aqueous extracts of *C. tinctorius* produced significant impact on dry weight of *O. punctata* seedlings. (Table 8). Data designated that substantial response was recorded with different concentrations of aqueous extracts and maximum (12.44 g) dry weight of *O. punctata* was measured at 1% concentration. The application of *C. tinctorius* extract at 0.25% showed lowest (2.86 g) dry weight of *O. punctata* (Table 8). In case of different plant parts, lowest (7.97 cm) dry weight of *O. punctata* was

recorded with the aqueous extracts of *C. tinctorius* stem whereas highest (12.52 g) dry weight examined with leaf aqueous extracts of *C. tinctorius*. The interaction among different concentration and plant parts was found non-significant. It is recorded that at 0.25% concentration with leaf extract of *C. tinctorius* produced the least (0.80 g) dry weight of *O. punctata*. Mubarak *et al.*, (2011) also supported that the differences in allelopathic potential of different plant parts were significant and extreme values of dry weight were recorded with flower as seedlings grow more on flower extract application than other opium plant parts extracts.

Allelopathic effect of *C. tinctorius* was observed on the emergence and initial seedling establishment of *O. punctata*. In this study the aqueous extracts of *C. tinctorius* exert inhibitory and also in some cases stimulatory allelopathic influence on root and shoot length, fresh and dry weight as well as on the emergence and initial seedling growth of *O. punctata* weeds that was depending upon concentration of aqueous extracts. This study was suggested that higher concentration (8%) with flower extract of *C. tinctorius* can be used for biological control of *O. punctata*.

Table-1: Mean emergence time of *O. punctata* as influenced by aqueous extract concentrations of leaf, stem and fruits of *C. tinctorius*.

Plant Parts	Mean emergence time							
	Concentration							Mean
	Control	0.25%	0.5%	1%	2%	4%	8%	
Leaf	3.92 ^{NS}	4.50	4.72	4.57	4.65	4.51	4.13	4.43 ^{NS}
Stem	4.25	4.68	4.22	4.30	4.69	4.25	4.27	4.38
Fruit	4.69	4.28	4.20	4.52	4.28	4.56	4.73	4.46
Mean	4.29 ^{NS}	4.49	4.38	4.47	4.54	4.44	4.37	

Effect of all the three parts are insignificant with each other at 5% probability level

HSD: Concentration= ^{NS}, Plant parts= ^{NS}, Concentration × plant parts= ^{NS}

^{NS}= Non-significant

Table-2: Emergence index of *O. punctata* as influenced by aqueous extract concentrations of leaf, stem and fruits of *C. tinctorius*.

Plant Parts	Emergence index (%)							
	Concentration							Mean
	Control	0.25%	0.5%	1%	2%	4%	8%	
Leaf	3.08a	3.14a	3.32a	3.28a	3.75a	3.56a	2.53a	3.24a
Stem	3.92a	3.67a	3.53a	3.25a	3.64a	2.72a	3.28a	3.43a
Fruit	3.69a	4.02a	3.33a	3.44a	3.47a	3.08a	1.92a	3.28a
Mean	3.56a	3.61a	3.39a	3.32a	3.62a	3.12a	3.58a	

Effect of all the three parts are insignificant with each other at 5% probability level
HSD: Concentration= ^{NS}, Plant parts= ^{NS}, Concentration × plant parts= ^{NS}
^{NS}= Non-significant

Table-3: Emergence percentage (%) of *O. punctata* as influenced by aqueous extract concentrations of leaf, stem and fruits of *C. tinctorius*.

Plant Parts	Emergence percentage (%)							
	Concentration							Mean
	Control	0.25	0.5%	1%	2%	4%	8%	
Leaf	86.67 ^{NS}	98.33	93.33	100	100	100	80	93.3^N
Stem	93.33	100	100	100	100	73.33	93.33	94.28
Fruit	100	93.33	93.33	100	100	93.33	80	94.29
Mean	93.33abc	95.56	95.56ab	100.0	100.00a	88.89	84.44	

Mean sharing the same letter did not different with each other at 5% probability level
HSD: Concentration = 4.46, Plant parts= ^{NS}, Concentration × plant parts= ^{NS}
^{NS}= Non-significant

Table-4: Time taken to 50% emergence of *O. punctata* as influenced by aqueous extract concentrations of leaf, stem and fruits of *C. tinctorius*.

Plant Parts	Time to 50% emergence (days)							
	Concentration							Mean
	Control	0.25%	0.5%	1%	2%	4%	8%	
Leaf	2.97	3.75	3.83	3.58	3.58	3.45	3.33	3.5a^{NS}
Stem	3.33b-e	3.75ab	3.42b-	3.50b-	3.33b-e	3.08de	3.25cd	3.38a
Fruit	3.58bcd	3.00e	3.33b-	3.50b-	3.33b-e	3.58bcd	4.19a	3.50a
Mean	3.30^{NS}	3.50^{NS}	3.53^{NS}	3.53^{NS}	3.41^{NS}	3.36^{NS}	3.59^{NS}	

Mean sharing the same letter did not different with each other at 5% probability level
HSD: Concentration= ^{NS}, Plant parts= ^{NS}, Concentration × plant parts = 0.461,
^{NS}= Non-significant

Table-5: Shoot length (cm) of *O. punctata* as influenced by aqueous extract concentrations of leaf, stem and fruits of *C. tinctorius*.

Plant Parts	Shoot length (cm)							
	Concentration							Mean
	Control	0.25%	0.5%	1%	2%	4%	8%	
Leaf	5.98de	5.93de	7.49bc	7.10bc	6.18cde	5.34de	4.51e	5.82
Stem	8.45ab	9.04a	8.28abc	9.80a	8.80ab	9.55a	8.72ab	9.06
Fruit	7.43bc	7.26bcd	8.63ab	7.90ab	7.91abc	7.23bcd	5.57de	7.21
Mean	7.28bc	7.41ab	8.13a	8.27a	7.63ab	7.37bc	6.27c	

Mean sharing the same letter did not different with each other at 5% probability level
 HSD: Concentration = 0.86, Plant parts = 1.297, Concentration × plant parts = 0.854

Table-6: Root length (cm) of *O. punctata* seedlings as influenced by aqueous extract concentrations of leaf, stem and fruits of *C. tinctorius*.

Plant Parts	Root length (cm)							
	Concentration							Mean
	Control	0.25%	0.5%	1%	2%	4%	8%	
Leaf	2.58cd	2.46cd	2.32cd	1.78d	2.00cd	1.68d	1.53d	1.91c
Stem	4.03ab	5.13a	3.27bc	3.55ab	4.80a	4.20ab	3.48ab	4.01a
Fruit	3.18bc	3.03bcd	3.61abc	2.88bc	3.40ab	2.90bcd	2.12cd	2.90b
Mean	3.26a	3.54a	3.40a	2.74a	3.40a	2.93ab	2.38b	

Mean sharing the same letter did not different with each other at 5% probability level
 HSD: Concentration = 0.638, Plant parts = 0.426, Concentration × plant parts = 1.734

Table-7: Fresh weight (g) of *O. punctata* seedlings as influenced by aqueous extract concentrations of leaf, stem and fruits of *C. tinctorius*.

Plant Parts	Fresh weight (g)							
	Concentration							Mean
	Control	0.25%	0.5%	1%	2%	4%	8%	
Leaf	89.17ab	80.33b	110a	90.67a	98.67ab	68.67b	54.72a	80.37
Stem	83.94ab	95.33a	94ab	68.00b	92.67ab	118.00	76.00a	87.72
Fruit	90.72ab	81.83a	97ab	98.67a	80.00ab	85.50a	75.44a	86.06
Mean	87.94a	61.50a	100.3	85.77a	90.44a	90.72a	68.72	

Mean sharing the same letter did not different with each other at 5% probability level
 HSD: Concentration = ^{N.S}, plant parts = ^{N.S}, Concentration × plant parts = 68.642
^{NS} = Non-Significant

Table-8: Dry weight (g) of *O. punctata* seedlings as influenced by aqueous extract concentrations of leaf, stem and fruits of *C. tinctorius*.

Plant Parts	Dry weight (g)							
	Concentration							Mean
	Control	0.25%	0.5%	1%	2%	4%	8%	
Leaf	15.67a	0.80a	0.65a	14.67a	15.33a	9.33a	7.61a	12.52
Stem	9.06a	3.25a	2.73a	11.33a	10.00a	4.77a	4.68a	7.97c
Fruit	9.39a	4.53a	5.83a	11.33a	8.67a	12.67a	4.56a	9.32b
Mean	11.37a	2.86a	3.07cd	12.44	11.33a	8.92b	5.62c	

Mean sharing the same letter did not differ with each other at 5% probability level
HSD: Concentration = 1.318, Plant parts = 3.305, Concentration × plant parts = ^{NS}
^{NS}=Non-Significant

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