Preservation of Morphine in Opium by Spraying Preservative Chemicals on Capsules and Latex of Opium Poppy (*Papaver somniferum* L.) cv. Dhawla Chotta

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Abstract

Field studies with the objective to prevent the loss of morphine in the latex oozing out after lancing the capsules of Opium poppy (Papaver somniferum L.) cv. Dhawla Chotta, were carried out at the University of Agriculture Research Farm, Makurdi, Nigeria, during 2003 and 2004 cropping seasons. Aqueous solutions of chelating agents, electrolytes, antioxidants, enzyme poisons and other preservatives alone and in combinations, when sprayed on fresh latex oozing out, arrested the deterioration of morphine in it. Spraying of aqueous solutions of chelating agent-I alone (a mixture of tartaric acid, citric acid, ascorbic acid and sodium tripolyphosphate) and in combination with sodium chloride on the surface of the capsule just before lancing starting at 115 days after sowing and spraying immediately after lancing on the latex oozing out gave the best results of highest morphine content (T_5, 13.78% ; T_3, 13.33%) which indicated that the deterioration of morphine in the latex was prevented significantly compared with the control (T_{10}, 9.72%). Opium poppy cv. Dhawla Chotta, a dwarf variety, yielded on an average, 63.6 kg ha^{-1} of opium at 70^0 consistence, 5.4 kg ha^{-1} of morphine in opium on dry matter basis and 900 kg ha^{-1} of poppy seeds. Agronomic practices to raise the opium poppy and collection of opium are described to increase the yield of opium and morphine ha^{-1}.

Keywords: Agronomic practices, Morphine, Preservation chemicals, Opium poppy.

Introduction

Opium poppy (*Papaver somniferum* L.) cv. Dhawla Chotta, a member of the family Papaveraceae, is a cash crop of high medicinal importance and yields the drug opium which is a stimulant, intoxicant, narcotic, used as sedative to induce sleep, relieve pain and relax spasms (Ramanathan, 1982). The latex obtained from the immature fruits of opium poppy is the source of opium (Kamath, 1992). Very little or no information is available on the agronomy and physiology of this crop in the southern guinea savanna zone of Nigeria.

Opium poppy is an erect herbaceous annual. It is important for pharmaceutical uses, hence, it is essential to maximize the yield of opium per hectare and collect it from the poppy capsule with the highest morphine content. Morphine (an alkaloid narcotic principle of opium) is contained in the fresh latex that oozes out on lancing the unripe capsules of *P. somniferum* L. After mid-day, morphine undergoes biochemical changes before the opium is collected the next morning after 2-3 hours exposure in the sun and hot air in the field (Knutson, 1995, Ramanathan 1980). Enzymes present in the fresh latex convert the morphine to other organic compounds and the conditions existing in the field accelerate this reaction (Antoun and Margaret 1974).

Application of chelating agents, electrolytes, antioxidants and enzyme inhibitors arrests the deterioration of morphine in the latex (Ramanathan, 1980). Preliminary study carried out at the Teaching and Research Farm of the University of Agriculture, Makurdi by spraying aqueous solutions of preservative chemicals on the fresh latex indicated that deterioration of morphine in the latex can be arrested (Obasi and Shittu, 2000). Consequently, field trials in the cropping seasons of 2003 and 2004 were conducted with the objective to prevent the loss of morphine in the latex oozing out after lancing the capsules of Opium poppy. The crop agronomy and plant protection measures are also described in order to increase the yield of opium ha^{-1}.

Materials and Methods

Experiments were conducted in three contiguous fields in 2003 and 2004 cropping seasons at the Teaching and Research Farm of the University of Agriculture, Makurdi (UAM) (7.4^{0}N, 8.37^{0}E, and 97m above sea level). The location falls within the southern guinea savanna agro-ecological zone of Nigeria. The soil of the experimental sites were well drained, sandy loam, low in organic carbon (0.19%) and available N (0.044%) and medium in available P (12.6 kg ha^{-1}) and available K(168.0 kg ha^{-1}), pH 8.0 and having available water capacity of 155 mm in the top 1m profile. Rainfall of 52.1 mm and 56.7 mm were received during the cropping period of 2003 and 2004, respectively.

The area of each field was 2500m^2 and each field was ploughed and harrowed two times and soil was well pulverised in June in both years. Each field was treated with Aldicarb (2-methyl-2-(methylthio) propionaldehyde 0-(methylcarbamoyl) oxime; 10G 1kg a.i. ha^{-1}, Farm yard manure (FYM) at the rate of 7.5 t ha^{-1} and 200 kg (4 bags) NPK 15:15:15 ha^{-1} at the time of land preparation and top dressed with 62.5 kg urea in two split doses at rosette and budding stages of growth at 50 days

and 80 days after sowing (DAS) respectively. Aldicarb was applied as a nematicide as well as a systemic insecticide and miticide.

The trials were laid out as a randomised complete block design in three replications and each plot measured 100 m^2. Seeds of opium poppy cv. "Dhawla chotta", obtained in 1997 from Department of Seed Science, Punjab Agricultural University, Ludhiana, India, were multiplied in isolated plots at UAM research farm and kept in storage for experimental use. The seeds were treated with Aldrex T at the rate of one satchet per 3 kg seed before planting on June 7th at the rate of 7 kg seeds ha^{-1}. Two seeds were sown per hill on the flat and spaced 75cm x 35cm. Thinning to one seedling per stand was done 25 days after sowing. The plots were weeded manually thrice before rosette, budding and lancing stages of crop growth i.e. at 45, 75 and 105 days after sowing. At 50 DAS and subsequently at 21 days intervals until 134 DAS, the plants were sprayed with vetox 85 W.P. insecticide at the rate of 1.5 kg ha^{-1} a.i., to control insect damage by black aphids (*Aphis craccivora* koch.) and fleabeetles (*Podagrica sjostedi* and *Podagrica uniforma*).

The lancing of capsules started 115 DAS and continued for 22 days till no more latex oozed out. The time of lancing was from 12 noon to 16 hours and the maximum field temperature during lancing ranged from 28^0C-$30^0C \pm 1^0C$. Aqueous solutions of preservative chemicals were prepared with 0.5 ml Teepol (surface active agent) per litre and sprayed gently for a moment (2 seconds) with a mist spray pump, first on the surface of the capsules just before lancing and second time over the latex oozing out immediately after lancing. Latex from each treatment was collected and safely stored separately in transparent polyethylene bags for the whole period of lancing and morphine per cent in it determined by the method described by the British pharmacopoeia (1988). From the control plots with no chemical spray, the latex was collected and stored in a similar way and the morphine content (%) in it was compared with that in the treated opium. Table 1 shows the preservative chemicals applied as treatments in the study.

Black salt is a UAM Laboratory product made on a small scale and does not have a uniform composition. The Black salt used in the present study have the composition shown in Table 2.

Statistical analysis was carried out on the basis of pooled mean of 2 years as outlined by Gomez and Gomez (1984) and Fisher's Least Significant Difference (F-LSD) test at 5% probability level was used to separate means according to Obi (2002).

Results and Discussion

The morphine content in the opium collected from no spray control plots were 10.22% and 9.72% (Tables 3, 4 and 5) which were the lowest compared with the morphine content in the latex collected from all other plots after spraying with preservative chemicals. All the preservative chemicals sprayed alone or in combination arrested the fall in the morphine content (Tables 3, 4 and 5). The morphine content in the opium after spraying of chelating agents alone (T_1, 13.22%), chelating agents plus black salt (T_6, 13.04%), sodium chloride (T_3, 12.96%) and chelating agents plus EDTA (T_4, 13.26%) (Table 3) indicated that the deterioration of morphine in the latex had been prevented by three units. The high yield of opium from 60 to 74 kg ha^{-1} (Tables 3 and 4) showed that the spraying of the chemicals on the capsules did not hinder the flow of the latex. With the high yield of opium and prevention of the biochemical reactions in the latex by spraying the chemicals, a maximum yield of 6 kg ha^{-1} of morphine was obtained (Tables 3, 4 and 5). Results in Table 5 showed that while the latex collected from the control plots with no spray had 9.72% morphine, the latex sprayed with chelating agent I (T_3), sodium chloride (T_1) and chelating agent I plus sodium chloride (T_5) had 13.33%, 13.19% and 13.78% morphine respectively. This result corroborates the earlier findings of the authors (Ramanathan, 1979, 1980) that the application of chelating agents and electrolytes alone or in combination arrests the deterioration of morphine in the latex.

Metals form an integral part of the metaloprotein enzymes which are organic catalysts. Enzymes remain dispersed in the liquid phase and are sensitive to pH and heat, most of them reacting between 25^0 and 38^0C. Rise in temperature of the substrate increases the velocity of the chemical reaction. The reactive groups of the amino acids in the enzymes accelerate the rate of biochemical reactions. Oxidation and reduction are performed by oxidoreductases. Fresh latex of opium poppy contains polyphenolases (Margaret 1971) and peroxidases (Murray, 1991). Polyphenolases change the milky latex of the poppy to brownish black solid (Ramstad, 1989). Peroxidase in the fresh latex forms H_2O_2 which is decomposed by catalase generating nascent oxygen. Normorphine and morphine N-oxide, which are decomposition products of morphine have been identified in opium (Trease and Evans, 1989). Chelating agents bind the metal ion co-factors in the enzymes and inhibit their chemical actions. Sulphur in SDDC acts as a poison and SDDC also binds the copper in the enzyme. Sodium chloride and black salt coagulate the enzymes destroying the interface action. TA, CA, AA, STPP and EDTA combine with amino acid groups and chelate with metal ions preventing the enzyme action (Miller *et al.*, 1973; Hussain, 1977). Ascorbic acid (AA) being a reducing agent counteracts the oxidation process set in by the enzymes. A cultivator having 2500m^2 of field and collecting opium upto five lancings will need for spraying, during the lancing period, 500g each of TA, CA, AA, STPP and SDDC and 5kg of sodium chloride to arrest the deterioration of morphine in the latex.

Yield of poppy seed was 901 kg ha^{-1} in field I; 897 kg ha^{-1} in field II and 902 kg ha^{-1} in field III. The average yield of the poppy seed from the three fields was 900 kg ha^{-1}.

Table 1: Chemical Composition of aqueous spray applied as treatments

S/N0.	Chemicals Sprayed with Teepol (Surfactant)
1.	Chelating agent 1: Tartaric acid (TA), Citric acid (CA), Ascorbic acid (AA) and Sodium Tripolyphosphate (STPP), 10g each in one litre of water.
2.	Chelating agent II: Sodium, diethyl dithiocarbamate (SDDC), 10g/litre.
3.	Sodium chloride: 50g/litre
4.	Black salt: 50g/litre
5.	Chelating agents I and II + TBHQ: 50mg/litre
6.	Chelating agents I and II + BHA: 50mg/litre
7.	Chelating agents I and II +AP: 50mg/litre
8.	Chelating agents I and II +Dichlone: 50mg/litre
9.	Chelating agents I and II +EDTA: 50mg/litre
10.	Chelating agents I and II +NDGA: 50mg/litre
11.	Chelating agents I + Sodium chloride: 50g/litre
12.	Chelating agents I + Black salt: 50g/litre

TBHQ = Tertiary Butyl Hydroxy Quinone, BHA = Butyl Hydroxy Anisole, AP = Ascorbyl Palmitate, EDTA = Ethylene Diamine Tetra Acetate, NDGA = Nor Dihydro Guaiaretic Acid.

Table 2: Composition of Black Salt

Composition	%
Moisture	0.26
Water insoluble (carbon and iron sulphide)	0.65
Sodium chloride	96.75
Sodium carbonate	0.44
Sodium sulphate	0.54
Sodium sulphide	1.36

Table 3: Effect of spraying preservatives on the capsules and latex of opium poppy (Field I)

Treatments	Chemicals sprayed	Aqueous solution rate	Yield of opium at 70^0 consistence (kg ha^{-1})	Morphine on dry matter (%)	Yield of morphine in opium on dry matter (kg ha^{-1})
T_1	Chelating agents I & II	1%	65.5	13.22 (21.44)*	6.08
T_2	Chelating agents I & II +dichlone	1%+50 mg/litre	64.3	12.94 (21.05)	5.83
T_3	Sodium chloride	5%	59.6	12.96 (21.08)	5.40
T_4	Chelating agents I & II +EDTA	1% + 50mg/litre	59.7	13.26 (21.34)	5.58
T_5	Black salt	5%	59.4	12.74 (21.24)	5.30
T_6	Chelating agents I & II + Black salt	1%+5%	69.1	13.04 (21.16)	
T_7	Chelating agents I & II + NDGA	1%+ 50mg/litre	61.5	12.70 (20.88)	5.47
T_8	Control (no spray)	-	60.8	10.22 (18.64)	4. 35
	SEM	-	3.2	0.42	0.37
	F-LSD (P < 0.05)	-	NS	1.28	NS

* *Figures in brackets are the angular transformed values, NS = Non significant.*

Table 4: Effect of Spraying Preservatives on the capsules and latex of opium poppy (Field II)

Treatments	Chemicals sprayed	Aqueous solution rate	Yield of opium at 70^0 consistence (kg ha^{-1})	Morphine on dry matter (%)	Yield of morphine in opium on dry matter (kg ha^{-1})
T_1	Chelating agents I & II+TBHQ	1% + 50mg/litre	71.2	12.17(20.42)*	6.06
T_2	Chelating agents I&II +BHA	1%+50 mg/litre	69.4	11.19 (19.53)	5.44
T_3	Chelating agents I&II +Black salt	1% + 5%	70.6	11.33 (19.67)	5.61
T_4	Chelating agents I & II + AP	1% + 50 mg/litre	74.4	11.64 (19.94)	6.06
T_5	Black salt	5%	70.4	12.11 (20.35)	5.99
T_6	Chelating agents I&II+ Sodium chloride	1%+5%	72.6	11.46 (19.79)	5.83
T_7	Control (no spray)	-	60.8	10.22 (18.64)	4.35
	SEM	-	1.93	0.17	0.20
	F-LSD (P<0.05)		5.88	0.52	0.61

* *Figures in brackets are the angular transformed values, NS = Non significant.*

Table 5: Effect of Spraying Preservatives on the capsules and latex of opium poppy (Field III)

Treatments	Chemicals sprayed	Aqueous solution rate	Yield of opium at 70^0 consistence (kg ha^{-1})	Morphine on dry matter (%)	Yield of morphine in opium on dry matter (kg ha^{-1})
T_1	Sodium chloride	5%	66.4	13.19 (21.24)*	6.14
T_2	Black salt	5%	53.3	12.29 (20.46)	4.60
T_3	Chelating agents I	1%	60.3	13.33 (21.38)	5.64
T_4	Chelating agents I & II	1%	59.4	12.77 (20.90)	5.31
T_5	Chelating agent I + NaCl	1%+5%	61.7	13.78 (21.08)	5.97
T_6	Chelating agents I+ Black salt	1%+5%	54.3	12.05 (20.27)	4.62
T_7	SDDC + NaCl	1%+5%	52.3	11.18 (19.49)	4.09
T_8	SDDC + Black Salt	1%+5%	66.0	11.15 (19.52)	5.15
T_9	SDDC + EDTA	1% + 50mg/litre	52.3	11.18 (19.49)	4.09
T_{10}	Control (no spray)	-	57.6	9.72 (18.37)	4.02
	SEM	-	2.68	0.37	0.33
	F-LSD (P<0.05)		7.96	1.11	0.98

* *Figures in brackets are the angular transformed values, NS = Non significant.*

Conclusion: The results of trials conducted showed that opium poppy on an average yielded 63.6 kg ha^{-1} opium at 70^0 consistence, 5.4 kg ha^{-1} of morphine and 900 kg ha^{-1} of poppy seeds. Spraying chelating agent I in combination with sodium chloride was most suitable to prevent the loss of morphine in the fresh latex of opium poppy.

References

Antoun, M. D. and Margaret, F. R. (1974). Methylating and demethylating enzymes in opium poppy (*Papaver somniferum* L.) latex. J. Pharmacy and pharmacology 26:112-113.

British Pharmacopoeia (1988). Opium. Pharmaceutical Press, London. p.331.

Gomez, K. A. and Gomez, A. A. (1984). Statistical Procedures for Agricultural Research (2nd ed.) John Wiley and Sons, New York. 680pp.

Hussain, S. (1977). Heavy metals in enzymes. Science Reporter, 14(2): 70-71.

Kamath, M.G. (1992). Dictionary of Indian Agriculture. Allied Publishers Ltd. 13/14 Asaf Ali Road New Delhi, 607pp.

Knutson, K. (1995). "Tropical Drugs". In: "The Science and Practice of Pharmacy". Remington J. T. ed. Mack Publishing Co. USA. Chap. 52 p.874.

Margaret, F. R. (1971). Polyphenolases in 1000g fraction of *Popaver somniferum* latex. Phytochemistry, 10:3021-3027.

Miller, R.J., Jolles, C. and Rapport, H. (1973). Morphine metabolism and nor-morphine in *Papaver somniferum*. Phytochemistry, 12:597-603.

Murray, R. D. H (1991). Progress in the chemistry of organic natural products. (Herz, W., Kurby, G. W., steglich, W. and Tamn C.H. eds). Springer volume 58 pp. 83 – 316.

Obasi, M. O. and Shittu, Z. (2000). Preliminary study on spray of preservative chemicals on fresh latex of opium poppy (*Papaver somniferum* L) Research. Monograph N0.8. Dept. Crop Production, Univ. of Agric Makurdi. pp. 1-8.

Obi, I. U. (2002). Statistical Methods of Detecting Differences Between Treatments Means and Research Methodology. Issues in Laboratory and Field Experiments. AP Express Publishers Ltd. 3 Obollo Road Nsukka, Nigeria. 117 pp.

Ramanathan, V. S. (1979). A Study on the deterioration of morphine in the fresh latex of *Papaver somniferum* L. United Nations Secretariat, ST/SOA/SER.J. 36:1-11.

Ramanathan, V. S. (1980). A Study on the deterioration of morphine and its reservation by chemicals in the fresh latex of opium poppy. Parts I, II, and III. Indian J. Agric. Res. 13(4): 229-237; 14(1): 6-12; 14(20): 82-90.

Ramanathan, V. S. (1982). Utilization of opium and its alkaloids in medicine. Cultivation and utilization of medicinal plants. Regional Research Laboratory. Council of Scientific Industrial Research, Jammu – Tawi, India. Vol. 11. pp. 159-172.

Ramstad, E. (1989). Enzyme and Opium. Modern Pharmacognosy, McGraw Hill Book Company, new York, pp. 343-407.

Trease, G. E. and Evans, W. G. (1989). Pharmacognosy, 13th edn. Balliere Tindall, London, pp. 215-306